Angiogenic Factors

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Within the past 2 years, several angiogenic factors have been fully purified, their amino acid sequences determined, and their genes cloned. These polypeptides include acidic and basic fibroblast growth factor, angiogenin, and transforming growth factors α and β. Other less well characterized angiogenesis factors have also been isolated, some of which are lipids. This article traces the discovery of the angiogenic factors and describes their possible significance in understanding growth regulation of the vascular system. When evaluated according to their putative targets, they appear to fall into two groups: (i) those that act directly on vascular endothelial cells to stimulate locomotion or mitosis, and (ii) those that act indirectly by mobilizing host cells (for example, macrophages) to release endothelial growth factors. In addition to their presence in tumors undergoing neovascularization, the same angiogenic peptides are found in many normal tissues where neovascularization is not occurring. This suggests that physiological expression of angiogenic factors is tightly regulated. In addition to the persistent angiogenesis induced by tumors, it now appears that a variety of nonneoplastic diseases, previously thought to be unrelated, can be considered as "angiogenic diseases" because they are dominated by the pathologic growth of capillary blood vessels.

Angiogenesis—Early Investigations

THE TERM "ANGIOGENESIS" WAS COINED IN 1935 TO DESCRIBE the formation of new blood vessels in the placenta (*1*). The technique of implanting a transparent chamber into a rabbit's ear made it possible to observe angiogenesis in a healing wound (*2*). In subsequent experiments, tumors were inserted into these chambers and tumor blood vessels observed (*3*). For two decades there was disagreement about whether tumors were supplied by existing vessels or neovascularization. Of those who favored neovascularization, some suggested that proliferating tumor cells induced the growth of new capillary blood vessels (*4*) [for review, see (*5*)]. Other workers argued that tumor neovascularization was only an inflammatory reaction. Still others claimed that tumors could make their own vascular channels lined by tumor cells. There was little if any evidence to suggest that tumor growth might be dependent upon capillary growth.

A hypothesis that tumors are angiogenesis-dependent. In studies initiated in 1963, it was found that tumors implanted into isolated perfused organs failed to grow beyond a few millimeters in diameter (*6*). However, when reimplanted into donor mice, these tumors grew rapidly, beyond 1 cm^3, and killed their hosts. In the mice, the tumors became vascularized; in the isolated perfused organs, they did not (*7*). Little significance was attached to this observation until it was appreciated that capillary endothelium in these organs gradually degenerated with prolonged perfusion (*8*). The isolated perfused organ provided the environment for a fortuitous failure of neovascularization which limited tumor growth.

On the basis of these studies, a hypothesis was formulated that "solid tumors are angiogenesis-dependent," and that "anti-angiogenesis" could be a potential therapeutic approach (*9*). In its simplest terms, this hypothesis can be stated: *Once tumor take has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries that converge upon the tumor* (*10*).

This idea was buttressed by experiments in which tumor cells were separated from their vascular bed to prevent neovascularization. Tumors stopped growing at a small size of 1 to 2 mm^3, but resumed rapid growth when vascularization was permitted (*11*). Furthermore, in vitro models of the prevascular state yielded dormant tumors (*12*). Histologic sections of tumors, with specially stained capillaries, strengthened the idea that tumor growth was linked to capillary growth (*13*). Tumor cells surrounded capillary blood vessels in a cylindrical configuration with a radius of 150 to 200 μm—equivalent to the diffusion distance for oxygen. DNA synthesis decreased with increasing distance of tumor cells from the nearest open capillary.

Isolation of a diffusible angiogenic factor from tumors. Two experiments in 1968 demonstrated that tumors could induce the growth of new capillary vessels despite separation of tumor cells from the vascular bed of the host by a Millipore filter (*14*). Here was presumptive evidence for the release of a diffusible tumor-derived angiogenic factor; however, an alternative explanation could not be excluded, degradation of an inhibitor by the tumor.

The first isolation of an angiogenic factor from tumors was reported in the early 1970s (*15*) and from conditioned media of transformed cells in 1976 (*16*). Other reports of tumor-derived angiogenic extracts followed (*17*). Purification of these factors was hampered, however, by the lack of suitable bioassays as well as inadequate knowledge of the components of the angiogenic process. It became apparent that the biology of angiogenesis would have to be understood in greater detail before biochemical analysis could succeed.

Development of new methods for studying angiogenesis. The phenomenon of angiogenesis was largely inaccessible to conventional experimental techniques. Four new methods for its study were developed in the mid-1970s: (i) The corneal micropocket technique permitted linear measurement of individual capillaries as they grew toward a tumor or an angiogenic substance implanted in the rabbit (*18*), mouse (*19*), or rat cornea. (ii) Biocompatible polymers were developed for the sustained release of angiogenic factors in vivo (*20*). (iii) The chick embryo chorioallantoic membrane was used to

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detect angiogenic activity of partially purified fractions from tumor extracts (*16, 21*). (iv) Vascular endothelial cells were cultured from umbilical vein (*22*), aorta (*23–25*) and capillaries (*26*), and used to guide the purification of endothelial cell growth factors.

Elucidation of the components of angiogenesis. These methods were used to dissect out the sequential events of capillary growth in vivo. It was found that new capillaries originated mainly from sprouting of small venules (*27*). Local degradation of the basement membrane of the parent venule was followed by movement of endothelial cells toward the angiogenic stimulus (*27*). The migrating endothelial cells elongated and aligned with one another to create a solid sprout. A lumen was then formed by a curvature that occurred within each endothelial cell (*27, 28*). Endothelial cell proliferation further increased the length of the sprout. Two hollow sprouts joined at their tips to form a loop, after which blood flow began. Pericytes positioned themselves along the base of the loop and new sprouts grew from the apex of the loop to continue the angiogenic process.

An understanding of the steps in capillary growth led to the development of in vitro bioassays based on three of these components of the angiogenic process: (i) enzymatic degradation of basement membrane (*29*), (ii) endothelial cell locomotion (*30, 31*), and (iii) endothelial cell proliferation (*23, 24, 26*).

Angiogenic Heparin-Binding Endothelial Cell Growth Factors

The availability of cultured endothelial cells and the appreciation that endothelial cell locomotion and proliferation were major components of angiogenesis encouraged efforts to isolate and purify endothelial cell growth factors. Among the first such growth factors to be described were basic fibroblast growth factor (FGF) isolated from brain (*32*) and endothelial cell growth factor (ECGF) isolated from hypothalamus (*33*). Retina (*34*), eye (*35*), and cartilage (*36*) were also identified as sources of endothelial cell growth factors. A breakthrough in the purification of endothelial cell mitogens came in 1983 as a result of the observation that an endothelial cell growth factor derived from rat chondrosarcoma had a marked affinity for heparin. Heparin-affinity chromatography was used to achieve a rapid two-step purification from chondrosarcoma of a homogeneous, cationic, 18,000 molecular weight, endothelial cell mitogen (*37, 38*). The chondrosarcoma-derived factor was angiogenic in both chick embryo and rat cornea bioassays (*39*).

Soon thereafter, many endothelial cell growth factors were found to have a strong affinity for heparin and the purification of these polypeptides was greatly facilitated by heparin-affinity chromatography (*40–45*). By 1985 the primary amino acid structures of two heparin-binding growth factors, basic FGF, a 146–amino acid polypeptide (*46*) and acidic FGF (*47–49*), a 140–amino acid polypeptide, had been determined. Basic and acidic FGF were found to be structurally related, having a 53% absolute sequence homology (*49*). By 1986, genes for basic FGF (*50*) and ECGF (*51*) (a precursor of acidic FGF) were cloned from libraries of complementary DNA (cDNA) and their respective nucleotide sequences determined.

Analysis of the various heparin-binding endothelial cell growth factors by several methods—heparin-affinity column elution profiles, protein sequences, immunological cross-reactivity and receptor binding—has greatly clarified the relation of these polypeptides to one another. Heparin-binding endothelial cell growth factors can be subdivided into two classes whose prototypes are acidic and basic FGF (*52*). The growth factors within a given class are either identical or represent multiple molecular weight forms of the same polypeptide.

One class of heparin-binding endothelial cell growth factors consists of anionic polypeptides that elute from heparin-Sepharose columns with approximately 1.0*M* NaCl. They have isoelectric points of 5 to 7 and molecular weights of 15,000 to 18,000. This class of heparin-binding growth factors has been found mainly in neural tissue and includes brain-derived acidic FGF (*40, 53*), ECGF (*42*), eye-derived growth factor II (*44*), and an acidic retina-derived growth factor (*43*). The other class of heparin-binding endothelial cell mitogens consists of cationic polypeptides that elute from heparin-Sepharose with 1.5*M* NaCl. They have isoelectric points of 8 to 10, molecular weights between 16,000 and 18,500, and appear to be identical to basic FGF or multiple molecular weight forms of it. The cationic class of heparin-binding growth factor appears to be more ubiquitous than the anionic. Polypeptides of the basic FGF class have been isolated from sources such as pituitary (*46*), brain (*40, 41, 54*), hypothalamus (*55*), eye (*44*), cartilage (*45*), bone (*56*), corpus luteum (*57*), adrenal gland (*58*), kidney (*59*), placenta (*60*), macrophages (*61*), chondrosarcoma (*38*), and hepatoma cells (*62*).

A human cDNA clone encoding ECGF, a precursor of acidic FGF, has been isolated from a library of human brain stem cDNA (*51*) and a bovine cDNA clone encoding basic FGF has been isolated from a pituitary cDNA library (*50*). The size of the human ECGF messenger RNA (mRNA) transcript is 4.8 kb; whereas 5-kb and 2.2-kb mRNA transcripts for basic FGF have been identified. In both genes the predicted amino acid sequences of the open reading frame begin with a methionine start codon followed by 154 amino acids. Amino acid sequence analysis of ECGF (*63*) and basic FGF (*64*) is in agreement with the gene sequence data and indicates that both are 154–amino acid polypeptides blocked at the amino terminus. It is now apparent that the lower molecular weight forms of acidic and basic FGF that were originally isolated and sequenced were truncated forms produced by proteinase cleavages at the amino-terminal end (*63–66*). These include the 140–amino acid form of acidic FGF (*47–49*) and the 146–amino acid form of basic FGF (*41, 46*). It is most probable that the various FGF-like molecules isolated from many sources are in reality multiple forms of acidic or basic FGF that have been enzymatically processed. The various tissues process acidic and basic FGF differently (*57–59, 63–66*). Whether this processing is physiologically significant or merely an artifact of purification is not clear.

Receptors for FGF have been identified by cross-linking techniques. Acidic FGF/ECGF receptors ranging in molecular weight from 135,000 to 150,000 have been found on capillary endothelial (*67*), aortic endothelial (*68*), and 3T3 cells (*68*), while receptors for basic FGF ranging from 125,000 to 145,000 have been found on BHK-21 cells (*69*) and bovine epithelial lens cells (*70*). In contrast to these reports of distinct acidic and basic FGF receptors, there is also a report of a single acidic and basic FGF receptor on the surface of myoblasts and 3T3 cells (*71*). Tyrosine phosphorylation has been reported for the acidic FGF receptor (*68*), but not for the basic FGF receptor (*69*). It remains to be determined whether basic and acidic FGF have distinct receptors. The extent and role of tyrosine phosphorylation of FGF receptors also needs further clarification.

Both classes of heparin-binding growth factor not only stimulate endothelial cell proliferation in vitro at 1 to 10 ng/ml but are angiogenic in vivo as well. Heparin-binding growth factors induce angiogenesis in nanogram amounts in the chick embryo chorioallantoic membrane (*39, 46, 47, 72*) and the cornea (*39, 72*). They also induce the formation of highly vascularized granulation tissue in sponges implanted subcutaneously in the rat (*73*).

An interesting question is raised by the fact that heparin-binding endothelial growth factors have been found in almost all normal tissues, yet endothelial proliferation in these tissues is exceedingly low with turnover times measured in years (*74*). Furthermore,

physiologic angiogenesis is infrequent and, when it does occur, seems to be restricted to females. How are these potent growth factors maintained in a functionally inactive state? One possibility is that heparin-binding growth factors are sequestered within their cells of origin and do not have access to vascular endothelial cells. There is little or no secretion of basic FGF by cultured cells (*62, 75*). By contrast, cells such as endothelial cells, which do not secrete basic FGF, do secrete platelet-derived growth factor (PDGF) (*76*). The lack of secretion of the fibroblast growth factors is consistent with the apparent absence of a classical signal peptide within these molecules as predicted by their gene sequences (*50, 51*). Thus, FGF could be a cell-associated angiogenesis factor that is released physiologically only under special circumstances, for example, during ovulation. Alternatively, FGF could be released after cell damage and thus play a role in tissue repair. The broad specificity of acidic and basic FGF for a number of target cells including endothelial and connective tissue cells and their wide distribution, makes these factors plausible candidates for being repair-mediating proteins.

Angiogenin

Angiogenin, a polypeptide first isolated from the conditioned medium of a human adenocarcinoma cell line, is a potent stimulator of angiogenesis at 0.5 to 290 ng in the chick embryo, and at 50 ng in the rabbit cornea (*77*). The polypeptide is a single chain with a molecular weight of 14,400 and a *p*I of 9.5 (*78*). Its gene has been cloned from a human liver cDNA library (*79*). Structural studies indicate that angiogenin has a 35% absolute sequence homology to a family of pancreatic ribonucleases. While angiogenin is inactive toward the more conventional substrates of ribonuclease such as wheat germ RNA, poly(C), poly(U), and RNA-DNA hybrids, it does cleave 28*S* and 18*S* ribosomal RNA to relatively large products of 100 to 500 nucleotides in length (*80*). It is not known whether the ribonucleolytic activity of angiogenin is involved in the mechanism of angiogenesis. Although both angiogenin and heparin-binding growth factors are potent stimulators of angiogenesis in the chick embryo and rabbit cornea, they are unrelated molecules. They differ in that angiogenin: (i) lacks sequence homology to either acidic or basic FGF; (ii) does not bind to heparin; (iii) is secreted by cells in culture and contains a signal peptide of 22 to 24 amino acids; and (iv) does not appear to be a mitogen for vascular endothelial cells. These properties suggest that angiogenin and the heparin-binding growth factors act by different mechanisms; however, the target cell specificity for angiogenin is not yet known. Therefore, its mechanism of action can only be speculative. It could cause the release of endothelial mitogens or chemoattractants from host cells, or it could mobilize macrophages to release these factors. Until the critical target for angiogenin is known, it may not be possible to explain its role in angiogenesis.

Transforming Growth Factors

Transforming growth factors (TGF) are polypeptides that when originally isolated from viral-transformed rodent cells were found to alter the phenotype of some normal cells to transformed cells (*81*). Thus, in the presence of these factors, fibroblasts pile up in culture and decrease their anchorage-dependence, but do not become neoplastic. In the past year, it has been discovered that these factors are also angiogenic in vivo. Two structurally distinct TGFs, TGF-α (*82*) and TGF-β, have been purified (*83*). Their structures have been determined by protein sequencing and cDNA cloning. TGF-α is a 50–amino acid polypeptide synthesized by transformed cells. It has a 35% homology to epidermal growth factor (EGF) and binds to the EGF receptor. Both TGF-α and EGF stimulate microvascular endothelial cell proliferation at 1 to 5 ng/ml (*84*). However, the angiogenic potency of TGF-α in vivo is an order of magnitude greater than EGF (0.3 to 1 μg of TGF-α versus 10 μg of EGF required to initiate angiogenesis). It is difficult to compare the angiogenic activity of TGF-α with that of the heparin-binding growth factors or angiogenin because the angiogenic activity of TGF-α has been tested only in the hamster cheek pouch instead of the chick embryo or cornea. Significantly higher concentrations of TGF-α were required to induce angiogenesis in the hamster cheek pouch than were necessary for heparin-binding growth factors and angiogenin to induce angiogenesis in the chick embryo or cornea. This difference could be due to diffusive losses. However, in our experience the hamster cheek pouch contains a relatively high density of resident macrophages and mast cells and permits neovascularization to be easily triggered by inflammatory substances. Therefore, it will be important to compare TGF-α with other angiogenic factors in the widely used chick embryo and cornea bioassays.

TGF-β is a 25,000 molecular weight homodimer (112 amino acids per chain), found in tumors and normal cells including kidney, placenta, and blood platelets. When injected into the nape of the neck in newborn mice at a dose up to 1 μg, TGF-β stimulates an increase in macrophages, fibroblasts, collagen production, and new capillary formation (*85*). A highly vascular granulation tissue forms at the site of the injection by 3 days. Neither EGF nor PDGF have that effect in the same bioassay. In the cornea, 1 to 10 ng of TGF-β induce a white cell infiltrate with opacification of the cornea followed by neovascularization (*86*). TGF-β inhibits proliferation of vascular endothelial cells in vitro (*87*). It is difficult to explain the apparent paradox between the in vitro and in vivo effects of TGF-β. However, TGF-β can stimulate or inhibit growth of certain non-endothelial cells depending on whether the cells are anchored or not and on the presence or absence of EGF (*88*). On the basis of these results, it has been suggested that TGF-β acts as a bifunctional regulator of cell growth in vitro. Furthermore, TGF-β is strongly chemotactic for macrophages in vitro, with a peak effect at approximately 0.04 to 0.4 p*M* which is equivalent to the chemotactic activity of f-Met-Leu-Phe* (*86*). It is possible that the macrophage chemotactic activity of TGF-β accounts for its angiogenic capacity in the chick and cornea. As the release of angiogenic factors from macrophages subsides, TGF-β could then suppress further endothelial proliferation.

Other Angiogenic Factors

A number of other factors (*17, 89*), not as well characterized in terms of structure and function as those described above, have been shown to be angiogenic [for a review, see (*90*)]. These partially characterized angiogenic factors can in part be placed in three categories: (i) low molecular weight endothelial mitogens, (ii) endothelial cell chemotactic factors, and (iii) lipids.

Low molecular weight angiogenic factors that are endothelial mitogens. Low molecular weight angiogenic factors (200 to 1000 m.w.) have been partially purified from rat tumors (*17, 91*), but have not been characterized as to their structure. In the absence of purified and characterized material, it remains difficult to evaluate the role of these factors in capillary growth.

Chemotactic factors. Angiogenesis factors that stimulate directional locomotion but not proliferation of endothelial cells have been isolated from wound fluids (*92*) and from monocytes (*93*). The

wound fluid factors appear to be polypeptides with molecular weights in the range of 2,000 to 14,000 but have not yet been completely purified. Angiogenesis has been induced in the cornea with partially purified extracts from wound fluids at 150 ng. It is not clear whether these wound fluid factors are perhaps products of macrophages in the wound or are being produced by another cell and are chemoattractants for macrophages. Macrophages that are properly activated can stimulate angiogenesis (*92, 94, 95*), and wounds that are deficient in macrophages also generally lack vascular ingrowth (*95*).

Lipids. Certain prostaglandins, such as PGE_1 and PGE_2, are angiogenic whereas prostaglandins of the A or F series are not (*96–98*). PGE_1 will stimulate angiogenesis in the cornea at 1 μg and PGE_2 stimulates angiogenesis at 0.2 to 20 ng in the chick embryo chorioallantoic membrane. It is not clear how prostaglandins induce capillary growth. However, prostaglandin levels are elevated in tumors, activated macrophages, wounds, and inflammatory exudates (*98*). Prostaglandins could act by mobilizing macrophages or by some as yet unknown mechanism. Certain uncharacterized polar lipids may also be angiogenic. They, along with a mixture of PGE_1 and PGE_2, constitute the major angiogenic activity secreted by adipocytes that have differentiated from 3T3 cells (*99*). These lipids are chemotactic to endothelial cells, but do not stimulate their proliferation.

Recently a non-dialyzable angiogenic factor that is also an endothelial mitogen was isolated from mixed cultures of T lymphocytes (*99a*).

Factors That Modulate Angiogenesis

Heparin. A number of observations over the past 10 years suggest a possible role for heparin in angiogenesis. Beginning with the report that mast cells that contain heparin accumulate at tumor sites before the ingrowth of new capillaries (*100*), the following findings have been made: (i) mast cells and mast cell–derived heparin stimulate locomotion of capillary endothelial cells in vitro (*31*); (ii) heparin augments angiogenesis induced by tumor in the chick embryo (*101*); (iii) protamine, a protein that binds avidly to heparin, inhibits the ability of mast cells and heparin to stimulate endothelial cell locomotion (*31*) and inhibits angiogenesis associated with embryogenesis, inflammation, and certain immune reactions (*101*); (iv) heparin potentiates the proliferative effect of acidic FGF on endothelial cells in vitro (*102*); (v) heparin inhibits angiogenesis in the presence of cortisone or hydrocortisone (*103*), or in the presence of certain corticosteroids which lack all of the functions usually attributable to hydrocortisone (*104, 105*); (vi) fragments of heparin that lack anticoagulant activity, such as hexasaccharides produced by enzymatic cleavage of heparin (*103*) or a synthetic pentasaccharide (*104*), also inhibit angiogenesis when administered with steroids; (vii) heparin-affinity chromatography is a powerful purification method for angiogenic FGFs (*38*), (viii) heparin increases the binding of ECGF to endothelial receptors (*106*); and (ix) heparin protects FGF from inactivation (*107*). Despite these interesting circumstantial associations of heparin, endothelial cells, growth factors, and angiogenesis, no conceptual framework has yet been proposed to explain the mechanisms underlying them.

Copper. There is a recurring theme in the literature that indicates that copper levels in tissue somehow modulate the intensity of the neovascular response to a given angiogenic stimulus. At this writing, there is no satisfactory model to connect these observations, but we have assembled them here because it may be productive to re-evaluate these data in the light of the newly described angiogenic polypeptides. (i) Copper ions augment endothelial locomotion in vitro (*108*). (ii) Copper levels increase in the cornea before capillaries penetrate it in response to an angiogenic stimulus such as PGE_1 (*97*). (iii) Rabbits on a copper-deficient diet are unable to mount an angiogenic response (to PGE_1), provided the serum copper level is lowered to about one-half the normal level or less (*97*). (iii) Ceruloplasmin, a copper-binding protein, is angiogenic in the cornea (although at a relatively high dose of 200 μg). Ceruloplasmin deprived of copper, however, is not angiogenic (*109*). (iv) Heparin can also act as a copper chelator (*110*), and very strong (Cu[II])-heparin interactions seem to occur at a small proportion of the anionic sites in the glycosaminoglycan (*111*). (v) Heparin becomes angiogenic in the cornea (at 20 μg) when it is complexed to copper, although heparin by itself is not angiogenic (*109*). (vi) The tripeptide Gly-His-Lys, which also is not angiogenic by itself in the cornea, becomes angiogenic when complexed to copper (*109*). (vii) Extracts of cornea in which any of these three copper-containing angiogenic effectors (copper complexes of ceruloplasmin, heparin, or Gly-His-Lys) have been implanted significantly increase the locomotion of capillary endothelial cells in vitro (*109*). (vii) There is a direct relation between copper-binding capacity and anticoagulant activity in heparin (*111*). These observations raise the question of whether copper and heparin interact to promote angiogenesis. Perhaps the affinity of the angiogenic factor FGF for heparin is copper-dependent. Beyond this speculation, it is too early to predict how copper functions as a modulator of angiogenesis (*112*).

Table 1. Biological activities of angiogenic factors.

Factor	Angiogenesis	Endothelial cell	
		Proliferation	Motility
Acidic FGF	Yes	Yes	Yes
Basic FGF	Yes	Yes	Yes
Angiogenin	Yes	No	ND*
TGF-α	Yes	Yes	ND
TGF-β	Yes	Inhibition	ND
Wound fluid	Yes	No	Yes
Prostaglandins	Yes	No	ND
Adipocyte lipids	Yes	No	Yes

*ND, not determined.

Discussion

Mechanisms of angiogenic stimulation. It is clear that a number of different factors can stimulate angiogenesis in vivo. However, they have quite different effects on capillary endothelial cell locomotion and proliferation in vitro, two of the key events necessary for the formation of new capillary blood vessels (Table 1). Some angiogenic factors stimulate endothelial locomotion or proliferation, or both. In contrast, others have no effect, or inhibit endothelial cell proliferation.

These results suggest that various angiogenic factors may operate either directly or indirectly when evaluated according to their putative targets. We have proposed that angiogenic factors that stimulate locomotion or mitosis of vascular endothelial cells in vitro have the vascular endothelial cell as their immediate target in vivo (*10*). Acidic FGF, basic FGF, and TGF-α are examples of such "direct" angiogenic factors. On the other hand, those angiogenic factors that have no effect on vascular endothelial cells in vitro can be categorized as acting by some "indirect" pathway in vivo. The details of such indirect pathways are not known but there are at least three possibilities, all of which are in the realm of speculation. It is possible that certain indirect angiogenic factors work by mobilizing

macrophages and activating them to secrete growth factors (*61*) or chemotactic factors (*92*) for vascular endothelial cells, or both. A second possibility is that indirect angiogenic factors cause the release of endothelial mitogens (for example, basic FGF) that are stored in the extracellular matrix (*75*). A third possibility is that indirect angiogenesis factors could release intracellular stores of endothelial growth factors (*75*).

When the different pathways for inducing angiogenesis are taken into consideration it is conceivable that some neovascular processes may utilize more than one pathway. For example, (i) tumor cells can synthesize their own angiogenic factors (*62*), (ii) some tumors can also attract macrophages and activate them to release angiogenic activity (*94*), (iii) other tumors, by virtue of their capacity to secrete specific collagenases and heparanases (*113*), may be able to release angiogenic factors stored in the extracellular matrix (*75*), (iv) some tumors may be capable of releasing more than one type of angiogenic factor, for example, FGF and TGF-α; and (v) certain tumors can release a vascular permeability factor that causes leakage of fibrinogen from postcapillary venules (*114*). The fibrin gel that subsequently forms in the extravascular space appears to play an important role in the buildup of a new capillary bed (*115*). Fibrin stimulates endothelial cell locomotion in vitro (*116*) and induces the influx of macrophages and new blood vessels when implanted in vivo (*117*). One can speculate that fibrin may provide a substratum for the elongation of capillary sprouts, and that fibrin degradation products may activate macrophages to secrete angiogenesis factors.

Mechanisms of physiologic inhibition of angiogenesis. Angiogenic factors, especially heparin-binding growth factors, have now been identified or isolated from a wide variety of normal tissues (*118*). This raises a central question. What prevents rampant capillary proliferation? In other words, why are the capillary endothelial cells of these tissues so quiescent under most normal conditions?

In addition to the possible intracellular controls that may restrict release of these factors (such as by sequestering FGF), there may also be extracellular physiologic inhibitors of angiogenesis. At the cellular level, recent evidence suggests that pericytes can suppress endothelial cell growth, and that this interaction seems to require direct contact between endothelial cells and pericytes (*119*). An example of such an inhibitor at the tissue level is found in cartilage (*120*). In the circulation, angiostatic steroids have been discovered that are natural metabolites of cortisone (*103, 104*). These compounds, such as tetrahydrocortisol, were previously thought to be biologically inactive. It is now known that they can cause capillary regression when administered at high doses in conjunction with heparin fragments. It is possible that at physiologic concentrations these steroids may act together with endogenous heparin-like molecules to restrain capillary growth.

Clinical implications. A picture is emerging that the process of angiogenesis is analogous to other processes, for example, blood coagulation, that must be maintained in a constant state of readiness for very long periods of time. The microvascular system seems to be designed to remain quiescent (*74*) without capillary growth, for prolonged periods (weeks for women, decades for men). A variety of controls appear to limit or prevent rampant capillary growth, just as there are physiological inhibitors that prevent intravascular clotting. However, on short notice the microvascular system appears capable of responding with rapid capillary growth to physiological demands such as ovulation, as well as to pathologic conditions such as wounds, chronic inflammation, certain immune reactions, and tumors. The rapidity of clot formation in a wound or in other pathological conditions is comparable.

There seems to be little or no biochemical difference between angiogenic peptides expressed by tumors and those found in normal tissues. Nor are there any essential morphological differences between the new capillaries that respond to a malignancy and the capillary growth that occurs during physiologic neovascularization. About the only demonstrable difference between tumor angiogenesis and other types of nonneoplastic angiogenesis is the great intensity and persistence of angiogenesis induced by tumors compared to other types of neovascularization. Tumors induce angiogenesis almost continuously until the neoplasm is eliminated or the host dies. None of the normal host mechanisms for controlling angiogenesis seem to limit tumor angiogenesis. This is quite different from nonneoplastic angiogenesis such as wound healing, where, for example, macrophage angiogenic capacity is shut off when tissue oxygen rises as new capillaries enter the wound (*121*).

We are beginning to recognize that the dominant pathology in many nonneoplastic diseases is persistent angiogenesis. These diseases occur in both males and females and are managed by physicians in many specialties of medicine and surgery. For example, diabetic retinopathy, retrolental fibroplasia, and neovascular glaucoma are some of the most frequent problems ophthalmologists must manage. In fact, abnormal neovascularization is one of the most common causes of blindness worldwide. Examples from other specialties include rheumatoid arthritis, where abnormal capillary growth can destroy joint cartilage; hemangiomas, in which abnormal capillary proliferation appears in newborn babies and may persist for up to 2 years, causing death from hemorrhage in some cases; angiofibromas, which appear in the nasopharynx usually in adolescents; psoriasis, where the excessive proliferation and shedding of epidermis may depend on abnormal capillary growth in the dermis; and capillary proliferation within atherosclerotic plaques which may bleed and contribute to sudden occlusion of coronary arteries (*122*). These pathologic states have previously been thought to be unrelated. We propose that they can be categorized as "angiogenic diseases."

It may also be helpful to consider a group of diseases in which capillary growth is slow or insufficient. These would include delayed wound healing (*95*), nonhealing fractures, and some congenital malformations such as hemifacial microsomia in which local or regional vascularization failed during fetal growth.

As we begin to understand more about the biological mechanisms of angiogenic factors it may be possible to develop therapeutic approaches for angiogenic diseases. Could the pathologic angiogenesis of diabetic retinopathy, rheumatoid arthritis, and the growth of tumors be suppressed by specific inhibitors of capillary growth? Can purified angiogenesis factors be administered in vivo, either locally or systemically, to accelerate the healing of wounds and fractures, or to increase neovascularization in the ischemic or infarcted heart? Can this be accomplished without initiating unwanted capillary proliferation in other parts of the body? These questions may now become the basis for new experimental approaches because of the availability of well-characterized angiogenic factors.

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