Annu. Rev. Med. 1998. 49:407–24

ANGIOGENESIS AND TUMOR METASTASIS

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KEY WORDS: blood vessels, invasion, angiogenesis inhibitors, tumor dormancy, cancer therapy

ABSTRACT

Angiogenesis, the recruitment of new blood vessels, is an essential component of the metastatic pathway. These vessels provide the principal route by which tumor cells exit the primary tumor site and enter the circulation. For many tumors, the vascular density can provide a prognostic indicator of metastatic potential, with the highly vascular primary tumors having a higher incidence of metastasis than poorly vascular tumors. Tumor angiogenesis is regulated by the production of angiogenic stimulators including members of the fibroblast growth factor and vascular endothelial growth factor families. In addition, tumors may activate angiogenic inhibitors such as angiostatin and endostatin that can modulate angiogenesis both at the primary site and at downstream sites of metastasis. The potential use of these and other natural and synthetic angiogenic inhibitors as anticancer drugs is currently under intense investigation. Such agents may have reduced toxicity and be less likely to generate drug resistance than conventional cytotoxic drugs. Clinical trials are now underway to develop optimum treatment strategies for antiangiogenic agents.

INTRODUCTION

Although it has long been recognized that most solid tumors contain large numbers of highly permeable blood vessels (1), the importance of these vessels in allowing the tumor to grow was generally unappreciated. In a startling series of papers in the early 1970s, Folkman laid out the principles that underlie the contemporary era of research in the field of tumor angiogenesis. He hy-

0066-4219/98/0201-0407$08.00

pothesized that the new vessels at the tumor site were not inconsequential but rather that they were absolutely required for expansion[1] of the tumor spheroid beyond a diameter of 1–2 mm, at which point diffusion of nutrients and waste products become rate limiting for continued development of the tumor (2). Folkman postulated that if new blood vessels were indeed essential for tumor growth, then inhibiting angiogenesis should inhibit tumor expansion. If the tumor vessels regressed after treatment, this should cause regression of the tumor mass back to an avascular 1- to 2-mm spheroid (3).

The first molecule definitively identified as a purified angiogenic factor was basic fibroblast growth factor (bFGF or FGF1) (4). This was followed by the identification of a large number of angiogenic factors (5) produced by tumor cells themselves and by accessory host cells such as macrophages, mast cells, and lymphocytes that may be attracted to the tumor (6–10). Although many of these factors have an occasional role in promoting tumor angiogenesis, recent attention has focused on members of the FGF (fibroblast growth factor) and VEGF (vascular endothelial growth factor) families as the most common tumor angiogenic factors. These factors have been described in detail in a number of excellent reviews (11–13). More recently, attention has turned to the isolation and characterization of angiogenic inhibitors that may have potential usefulness as anti-tumor agents (14, 15).

ANGIOGENESIS AS AN ESSENTIAL COMPONENT OF TUMOR METASTASIS

The early studies and theories regarding tumor angiogenesis focused on the effects of the new blood vessels on tumor expansion and the potential for causing tumor regression by using angiogenic inhibitors. What has been less well appreciated is that angiogenesis is a critical component of tumor metastasis and that highly vascular tumors may have the potential to produce metastases at a higher rate than less angiogenic tumors. Beyond its effects on tumor expansion, perhaps the most important way in which angiogenesis can facilitate tumor metastasis is by providing an efficient route of exit for tumor cells to leave the primary site and enter the blood stream. Angiogenesis enhances entry of tumor cells into the circulation by providing an increased density of immature, highly permeable blood vessels that have little basement membrane and fewer intercellular junctional complexes than normal mature vessels (12). As many as 2×10^6 mammary carcinoma cells can be shed into the circulation from a 1-

[1]The term tumor expansion is used in place of tumor growth in this context to indicate a measureable increase in tumor mass. In avascular tumors, cell growth takes place but is balanced by cell death so that there is no net increase in tumor size.

cm primary tumor each day (16), though few of these ever form metastases. The number of metastases formed is generally proportional to the number of tumor cells shed. Consequently, a decrease in angiogenesis in a given metastatic tumor should produce a decrease in the number of tumor cells shed into the circulation and a corresponding decrease in the number of metastatic colonies that arise downstream.

The correlation between angiogenesis and tumor metastasis can be seen clearly in experiments in which animals with established primary tumors are treated with angiogenesis inhibitors. Decreased vascularity of the primary tumor is virtually always associated with decreased formation of metastatic colonies. This was first shown in early experiments with the angiogenic inhibitor protamine sulfate, although the toxicity of this compound made interpretation of these results difficult (17). Similar results have since been shown with nearly every angiogenic inhibitor discovered, regardless of its mechanism of action. Virtually all well-characterized angiogenic inhibitors inhibit tumor metastasis. The list of angiogenic inhibitors that also inhibit tumor metastasis includes the angiostatic steroids (18), thalidomide (19), the fumagillin analog TNP-470 (20–22), thrombospondin (23), angiostatin (24), endostatin (25), platele factor 4, (26), and the synthetic protease inhibitor BB94 (27).

ANGIOGENESIS AS AN INDICATOR OF METASTATIC POTENTIAL IN HUMAN TUMORS

Angiogenesis has been implicated in affecting outcomes in human cancer. Angiogenic factors are found within tumors as well as in such body fluids as serum, urine, and ocular fluids (28–32). Several studies show a correlation between production of angiogenesis factors and relapse, metastasis, and poor prognosis in human cancer patients. Renal cancer patients with high levels of the angiogenic factor bFGF in their primary tumors have a poorer survival rate than do patients with lower bFGF levels, suggesting that increased angiogenesis due to bFGF production may lead to increased metastatic potential and consequently decreased survival (33). In breast cancer, VEGF production correlates with early relapse (34). In addition, it appears that tumors that produce multiple angiogenic factors show increased rates of primary tumor expansion (35).

Perhaps the most compelling correlation between angiogenesis and tumor metastasis has been in the large number of studies in which vascular density of a tumor has been correlated with metastasis and with patient outcome. In the first study of this type, Weidner et al (36) showed a direct correlation between the vascular density (number of vessels per high-powered field) and the likelihood of metastasis in human breast cancer patients. The implication of this

study is that vascular density can function as an independent prognostic variable in breast cancer. Since this report was published, a large number of groups have repeated this study with breast cancer patients and most have confirmed the initial correlation (reviewed in 37–40). This finding is not limited to breast cancer but has been extended to several other tumors, including carcinoma of the prostate (41, 42), lung (43, 44), stomach (45), cervix (46), and ovary (38), and in squamous cell carcinoma of the head and neck (47). Thus, for many tumors, increased vascular density is indicative of increased metastasis and decreased survival.

ANGIOGENIC CONTROL OF METASTATIC COLONY SIZE

Micrometastases

It is common in experimental models of metastasis to find organs in which there are no visible metastases, but histological examination reveals many micrometastases consisting of individual tumor colonies of very small diameter. Careful examination often reveals that such tumors are avascular. They can survive for long periods of time without further expansion. Although the term dormancy has been applied to such tumors, it is important to realize that the rate of cell proliferation in such tumors is virtually the same as in rapidly expanding tumors. The difference is that the dormant tumors have increased numbers of dying cells. This has been shown by the work of Hanahan and colleagues, who have followed tumor development in transgenic mice that develop pancreatic islet tumors. Here, the initial phase of tumor growth is characterized by avascular tumors that maintain a small diameter for a period of weeks until an angiogenic "switch" is activated and the tumors become vascular and begin to expand in size (48). Treatment of such mice with angiogenesis inhibitors blocked formation of these tumor colonies (49). The role of cell death in metastatic dormancy has also been shown by the work of Holmgren et al (50), who demonstrated that dormant, avascular tumors caused by the presence of an angiogenic inhibitor have increased numbers of cells undergoing apoptosis or programmed cell death. These results indicate that dormant micrometastases differ from growing tumors in that the dormant tumors have increased cell death rates secondary to decreased angoigenesis.

Evidence for Angiogenic Control of Tumor Dormancy

In an elegant series of experiments, O'Reilly et al recently demonstrated that certain murine and human tumors can produce circulating angiogenic inhibitors that act downstream to suppress metastatic expansion at the secondary site

(24, 25). In animals carrying implanted tumors that produce circulating angiogenic inhibitors, growth at the primary site is allowed because the angiogenic balance is positive. Here, short-lived angiogenic stimulators have greater activity than the local levels of inhibitor. At the distant secondary site, however, the balance is reversed. Long-lived inhibitors from a large primary tumor can swamp the stimulatory activity of angiogenic factors produced by the small metastatic colonies. This can lead to the establishment of dormant micrometastases. When the inhibitor-producing primary tumor is removed, the metastases become vascularized and grow rapidly (24, 25).

ANTIANGIOGENIC THERAPY FOR METASTATIC TUMORS

One of the limitations of conventional cancer therapies is that they are often of limited use in causing regression of tumors that have undergone distant or widespread metastasis. One of the potential advantages of antiangiogenic therapy is that, at least in experimental animals, antiangiogenic agents can cause regression of metastatic lesions and also prevent their dissemination (51, 52). It is of interest that some agents—including taxol, tamoxifen, and adriamycin, which are already in clinical use as anti-tumor agents—are being found to have antiangiogenic activity (53–55). Teicher and colleagues have reported that the combination of conventional chemotherapeutic agents with antiangiogenic agents gave significantly better results in reducing tumor metastases than was found with either agent alone (56, 57). Although clinical trials with a few antiangiogenic agents such as the fungal drug analog TNP-470 (58) and the calcium signaling inhibitor carboxyamidotriazole (59) are currently in progress, few have been completed and many promising agents such as angiostatin and endostatin have not yet entered clinical trials. It is likely that many additonal antiangiogenic agents will be identified as a result of the intense screening now taking place to identify new drugs of this type (60).

At this early point, it appears that long-term, low-dose therapy with antiangiogenic agents may give the most promising results, as there may be some lag time between administration of an antiangiogenic agent and the reversal of tumor progression. It will be important for clinicians using antiangiogenic agents as anticancer drugs to rethink some of the paradigms that have developed from use of conventional chemotherapeutic agents that are designed to kill the largest number of tumor cells in the shortest possible time. Antiangiogenic agents are more likely to be used as chronic, low-dose therapies where the intention is to cause gradual tumor regression and then provide long-term prophylaxis to prevent widespread metastatic growth.

NATURALLY OCCURRING ANGIOGENIC INHIBITORS

Thrombospondin

A considerable amount of research has been conducted on the antiangiogenic properties of the extracellular matrix molecule thrombospondin. The original work on this field was conducted by Bouck and colleagues, who correlated the antiangiogenic activity of thrombospondin with the expression of a tumor suppressor gene (61, 62). Thrombospondin was soon confirmed to be an inhibitor of endothelial cell proliferation (63), motility (64), and morphogenesis (65). The initial correlation of thrombospondin production with tumor suppression was clarified when it was reported that the well-characterized tumor suppressor P53 could repress angiogenesis by up-regulating the production of thrombospondin or other inhibitors in certain tumor cells (66–68). In a compelling recent study, the expression of thrombospondin was shown to be inversely related to P53 expression and to angiogenesis in human bladder cancer specimens (69).

Interferon

The antiendothelial activity of interferon has been known for some time. An early report from our laboratory demonstrated that interferon could inhibit the migration of capillary endothelial cells, a critical step in angiogenesis (70). Interferon has subsequently been shown to have some in vivo antiangiogenic activity (71), but it is not sufficiently potent to be able to cause regression of many tumors when used alone. One potential mechanism of interferon action may be to block the production or efficacy of angiogenic factors produced by tumor cells (72). Some vascular tumors are more sensitive to the inhibitory activity of interferon. Hemangiomas, large benign tumors comprised predominantly of endothelial cells, are particularly sensitive to treatment with interferon. Treatment with α-interferon is one of the first clinically successful treatment protocols for patients with proliferating hemangiomas (73–76). Treatment is chronic and of long duration, often lasting a year or longer, but α-interferon is relatively nontoxic and provides the only documented treatment for this potentially disfiguring, sometimes fatal disease.

Metalloproteinase Inhibitors

Invasive events require both active cell migration and the ability to cause limited degradation of the connective tissue in order to allow passage of the tumor cells through tissue. This is accomplished, in part, by the activity of metalloproteinases that are sequestered on the tumor cell surface and concentrated at the leading edge of the tumor cell (77). Called matrix metalloproteinases

(MMPs) because of their ability to degrade extracellular matrix, these enzymes inhibit both angiogenesis and tumor metastasis (78).

Naturally occurring MMP inhibitors, known as TIMPs (tissue inhibitors of metalloproteinases), have been found in a variety of cells and tissues. All members of the TIMP family inhibit angiogenesis (79, 80). In addition, a naturally occurring angiogenesis inhibitor isolated from cartilage has TIMP-like domains (80a). TIMPS also inhibit tumor growth (81) and metastasis (82–84). Although the mechanism whereby TIMPs inhibit angiogenesis and metastasis would appear to be their ability to suppress matrix degradation (85), other cellular effects of the TIMPs make interpretation more difficult, because these protease inhibitors can also directly block proliferation and migration of both tumor cells and endothelial cells (78, 80, 86, 87) in vitro. The combined activities of this class of inhibitor make them potent anti-tumor agents.

SYNTHETIC ANGIOGENESIS INHIBITORS

Synthetic Protease Inhibitors

The realization that metalloprotease inhibitors could be useful antagonists of the invasive processes that occur in both angiogenesis and metastasis has prompted the development of synthetic protease inhibitors that might be useful clinical therapeutic agents. An early example of this class of inhibitor is BB94 (88), or batimastat (89), showing potential anti-tumor growth (90, 91), antiangiogenesis (89), antimetastatic activity in experimental tumors (92, 93), and relatively low toxicity in preliminary clinical trials (88). Although the rationale for developing such agents was to have better anti-invasion agents that would block events downstream of tumor proliferation, it now appears that such agents can block cell proliferation and expansion of both primary and secondary tumors. Thus, their reported clinical utility may be attributable as much to their direct anti-tumor proliferative activity as to their anti-invasive activity.

Anti-adhesive Peptides

Cell adhesion is required in many steps of the angiogenic process, including endothelial cell migration, proliferation, and morphogenesis. Although several adhesive molecules are likely to be involved in this process, recent work from Cheresh and colleagues has focused on the $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins as important mediators of angiogenesis (94). Angiogenesis and concomitant tumor growth can be inhibited by anti-integrin antibodies (95) as well as by peptide antagonists that block the interaction of these integrins with their extracellular matrix ligands (96). In the presence of these inhibitors, endothelial cells in new vessels undergo apoptosis, whereas the cells in preexisting mature vessels are unaffected (96).

An elegant application of this technology has been reported in a recent publication by Pasqualini and coworkers (97), in which cyclized RGD-containing peptides that inhibit integrin function were displayed on bacteriophage. When the phage were inoculated into experimental animals, they localized preferentially in the tumor blood vessels, presumably as a result of interactions with the endothelial integrins that have been up-regulated during angiogenesis (97). The authors speculate that this technology could be employed to target antiangiogenic agents to sites of neovascularization, such as tumors. Thus, the finding of specific adhesive molecules on the surface of angiogenic endothelium has led to potential new methods of angiogenesis inhibition. Such antiangiogenic strategies that directly target tumor endothelium might be expected to have very low toxicity due to their limited accessibility beyond the vasculature.

TUMOR-DERIVED INHIBITORS

Angiostatin

It now appears that tumor cells make both stimulators and inibitors of angiogenesis and that the balance between these factors dictates the degree of angiogenesis both locally and at distant sites. Angiostatin was the first molecule specifically isolated as a potential tumor-derived angiogenesis inhibitor (24). Isolated from the urine of mice carrying large Lewis lung carcinoma tumors, angiostatin was found upon sequencing to represent a specific fragment of the clotting cascade protease precursor plasminogen. This is interesting because it provides an example of a specific precursor that can be cleaved in different ways to provide both effectors of the clotting cascade (plasmin) and of the angiogenic process (angiostatin). It is not thought that angiostatin itself is produced by tumor cells but rather that certain tumors can produce or activate proteases capable of generating angiostatin from circulating plasminogen. A serine protease that specifically cleaves angiostatin from plasminogen is produced by prostate cancer cells (98). Angiostatin has been reported to be an endothelial-specific inhibitor of both endothelial cell proliferation and migration and can act as a circulating angiogenesis inhibitor that suppresses angiogenesis at downstream sites distant from the tumor. Angiostatin treatment of tumor-bearing mice causes regression of the primary tumor and prevents vascularization and growth of metastatic colonies.

Endostatin

Endostatin is the second published example of a tumor-derived angiogenesis inhibitor. Like angiostatin, endostatin is a fragment of a larger molecule, in this case collagen XVIII, a novel collagen frequently found near blood vessels

(25). Endostatin is reported to be a highly active endothelial-specific inhibitor that inhibits microvascular endothelial cell proliferation at doses of 100–500 ng/ml. Endostatin inhibits primary tumor growth as well as establishment and growth of metastases. Combinations of endostatin and angiostatin are synergistic and can result in complete tumor regression with no regrowth after treatment is discontinued (J Folkman, personal communication). The finding that both angiostatin and endostatin are fragments of larger precursor forms that have no angiogenic effector activity themselves suggests that the natural control of angiogenesis is much like that of repair processes such as blood clotting, in which latent precursors become activated by proteolysis only when needed. Other angiogenic effectors that are also derived from larger molecules include fragments of the extracellular matrix molecules SPARC (99, 100) and thrombospondin (101), a plasmin-generated fragment of platelet-factor 4 (102), and the 16-kDa N-terminal fragment of prolactin (103, 104).

PHARMACOLOGIC AGENTS THAT INHIBIT ANGIOGENESIS

AGM1470/TNP470

The initial observation by Ingber et al (20)—that the fungal antibiotic fumagillin was a potent angiogenesis inhibitor—gave rise to the testing of synthetic analogs of fumagillin for their relative antiangiogenic activty. Among the most active of these analogs was O-chloroacetylcarbamoyl fumagillol, referred to first as AGM-1470 and more recently as TNP-470. This agent inhibits endothelial cell proliferation in vitro and angiogenesis in vivo (20). TNP-470 has been more widely studied in human trials than any other newly developed antiangiogenic compound (60). The drug is being used both as a primary antitumor treatment and also as a sequel to other treatments. Preliminary results suggest that long-term (>1 year) treatments with TNP-470 are optimal and that the drug should not be stopped early if the tumor appears to progress, because the effects of the agent take some time to be translated into tumor stasis or regression.

Thalidomide

In a surprising recent finding, D'Amato and colleagues reported that the drug thalidomide has potent antiangiogenic activity (19). Originally used as a sedative, thalidomide's use was discontinued when it was found to be a potent teratogen, causing serious birth defects, especially affecting limb development. The antiangiogenic activity of thalidomide now provides a potential mechanism for this teratogenic activity, since limb development is especially sensi-

tive to the vascular density of the growing limbs. Thalidomide is relatively nontoxic, however, when taken by nonpregnant adults and is now being tested in early (phase 2) clinical trials as a potential anticancer agent as well as a treatment for vascular eye diseases such as diabetic retinopathy, blindness of prematurity, and macular degeneration.

CAI

Carboxyamidotriazole (CAI) is a calcium channel inhibitor that blocks tumor cell migration and proliferation and has antiangiogenic activity. CAI retards metastasis in experiment animals and has completed phase I clinical trials in cancer patients. Published results from these trials showed disease stabilization in 49% of the patients who had disease progression before starting CAI treatment (59). Further evaluation of this intriguing multimodal antitumor agent is currently underway.

POSSIBLE TREATMENT STRATEGIES FOR ANTIANGIOGENIC THERAPY OF METASTATIC TUMORS

If it were true that antiangiogenic agents could be useful in causing tumor regression, how might they be best used? The most commonly held theories of angiogenesis predict that, in most cases, some tumor may remain after a course of antiangiogenic treatment. That is, the tumor would regress until it reached a small-enough size (<2 mm thick) to be no longer dependent on angiogenesis. If this took place, the tumor might be expected to stay at this size as long as the treatment were continued. If, however, the antiangiogenic treatment were discontinued, the tumor would be expected to regrow. For these reasons, and also because antiangiogenic therapy usually will not generate resistant populations, it has been suggested that antiangiogenic agents might best be delivered in two ways. First, they could be administered at the same time as conventional chemotherapy. This type of combination might have significant advantages over combination cytotoxic therapy, since there will be two completely different modes of action utilized at the same time (56, 57). In addition, antiangiogenic treatments may be given over long periods of time after the termination of cytotoxic chemotherapy. The intent here is to stimulate further tumor regression while keeping existing micrometastases from breaking through and preventing new metastases from arising. It is also conceivable, depending on the eventual side effects of such treatment, that antiangiogenic agents could be given prophylactically to individuals known to be at high risk for developing new or recurrent tumors.

It is clear we are entering the age of exploration of angiogenesis inhibition for the treatment of both primary and metastatic tumors, ocular vascular disease, and possibly chronic inflammatory disease. Although several important candidate inhibitors have emerged and are effective inhibitors of angiogenesis and of tumor growth and metastasis in animals, none have been extensively tested yet in humans. Consequently, we do not know which of these agents will combine the greatest efficacy with the least toxicity. It is unlikely that the most effective angiogenic inhibitor has yet been discovered. There is, therefore, considerable opportunity for the discovery of natural or synthetic new inhibitors.

Potential Side Effects of Antiangiogenic Therapies

The potential side effects of each angiogenic inhibitor will need to be studied in great detail. Although it is expected that angiogenic inhibitors will have considerably less toxicity than conventional chemotherapeutic agents have, there has been little experience with administration of such agents in humans. Because of the possibility of using antiangiogenic treatments prophylactically to prevent tumor dissemination or to maintain tumor dormancy, it will be essential to study toxicity of these agents over long time periods. Although little angiogenesis takes place in normal adults, some potential areas of concern include wound healing and other normal repair processes that may depend on neovascularization. Extra caution may also be needed when treating women in their childbearing years. Angiogenesis has a critical role in ovarian development, and angiogenic inhibitors have potential contraceptive activity (105). Furthermore, at least one known teratogen, thalidomide, functions as an angiogenesis inhibitor and may possibly affect limb development via its antiangiogenic activity (19). Consequently, angiogenic agents should be tested for teratogenic properties in addition to ascertaining their safety in adults.

Can Total Tumor Regression be Achieved by Administration of Angiogenic Inhibitors?

In the original hypothesis formulated by Folkman in the early 1970s of angiogenic control of tumor growth (2, 3), it was proposed that tumor growth was limited by diffusion to a size of 1–2 mm unless additional blood vessels were recruited to the tumor site. The arrival of these new blood vessels allowed continued three-dimensional expansion of the tumor. Antiangiogenic treatment can reduce a tumor mass back to its avascular size, but it may not completely eliminate tumors that regress to sizes no longer dependent on increased vascularity. Consequently, antiangiogenic agents may be given in conjunction with other types of direct anti-tumor therapy so that tumors reduced to small size by

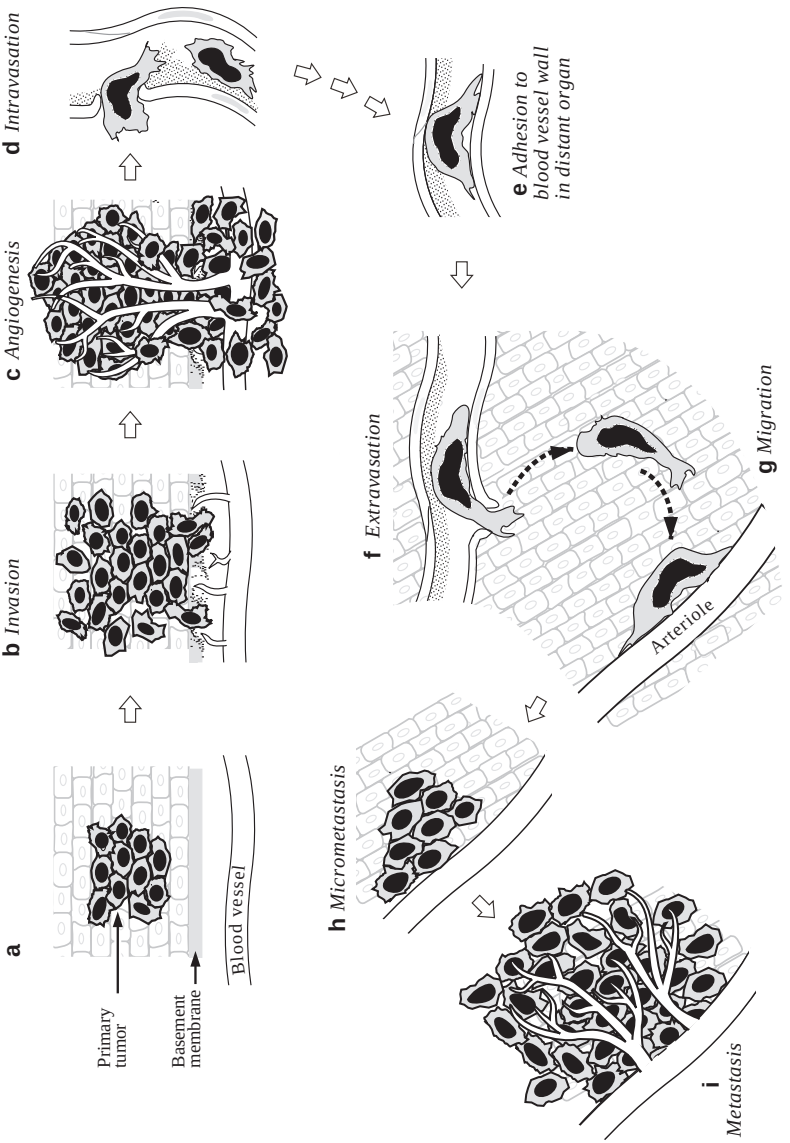
antiangiogenic therapy can be eliminated by cytotoxic agents. The antiangiogenic agents may also be useful for prolonged treatment to prevent regrowth of dormant micrometastases. There are, however, sporadic reports of tumors that have been completely eliminated by antiangiogenic therapy alone. The combination of angiostatin and endostatin can lead to complete tumor regression in mice, presumably through an antiangiogenic mechanism (J Folkman, personal communication). It is therefore possible that strategies will emerge that use antiangiogenic therapies as the primary antitumor therapy, without the addition of cytotoxic agents. Future clinical trials are necessary to see whether this is a viable strategy in human cancer patients.

SUMMARY

Three decades of intensive research on the role of angiogenesis in promoting tumor growth and metastasis have left us with several important conclusions (Figure 1).

We now know that both expansion of the primary tumor and metastasis to distant organs depend critically on the formation of new blood vessels that provide increased availability of oxygen and nutrients to the tumor as well as the most important route of exit from the primary tumor into the blood stream. Large numbers of tumor cells can be shed daily into the angiogenic blood vessels that have been recruited to the tumor. Large solid tumors contain cells that release one or more angiogenic factors such as basic fibroblast growth factor and vascular endothelial growth factor. The best strategies for inhibiting angiogenesis repress the ability of the endothelial cell to participate in the angiogenic process rather than prevent tumor cells from producing one particular angiogenic factor, since the plasticity of the tumor cell population generally allows the development of cells that produce other angiogenic factors and thus the tumor may become resistant to treatment. In contrast, treatments that directly target the endothelial compartment by inhibiting components of

Figure 1 Vascular components of tumor metastasis. The steps of the metastatic pathway that involve interactions with blood vessels: *(a)* small primary tumors (<2 mm) remain avascular until they *(b)* invade the local epithelial basement membrane. If the tumor cells produce angiogenic factors *(c)* angiogenesis will occur, allowing expansion of the primary tumor. *(d)* The new blood vessels provide a route of entry into the bloodstream and the tumor cells circulate until they die or *(e)* attach specifically to endothelial cells in the vessels (usually venules) of downstream organs. *(f)* The tumor cells extravasate through the vessel wall and then *(g)* migrate to sites proximal to arterioles where their growth is enhanced. *(h)* Micrometastases can remain dormant for extended time periods during which angiogenesis is suppressed. *(i)* Initiation of angiogenesis at the secondary site releases the metastatic colonies from dormancy and allows rapid growth.


a
Primary tumor
Basement membrane
Blood vessel
b Invasion
c Angiogenesis
d Intravasation
e Adhesion to blood vessel wall in distant organ
f Extravasation
g Migration
Arteriole
h Micrometastasis
i Metastasis

the angiogenic process, such as endothelial cell adhesion or migration, may generate tumor treatments that do not lead to the generation of drug resistance. Angiogenesis inhibition can lead to tumor regression and, in some cases, to complete elimination of the tumor. Results from early phase clinical trials employing first-generation angiogenesis inhibitors such as TNP-470 suggest that unlike conventional chemotherapeutic agents that are generally employed at high doses for relatively short time periods, angiogenic inhibitors may be best employed using low doses over months to years as a potential means to prevent dormant micrometastases from entering a rapid growth phase. The additional clinical testing of newly identified angiogenic inhibitors using a variety of delivery strategies is eagerly awaited.

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