The Contribution of Angiogenesis to the Process of Metastasis

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Abstract: The role of angiogenesis in tumor growth has been studied continuously for over 45 years. It is now appreciated that angiogenesis is also essential for the dissemination and establishment of tumor metastases. In this review, we focus on the role of angiogenesis as a necessity for the escape of tumor cells into the bloodstream and for the establishment of metastatic colonies in secondary sites. We also discuss the role of tumor lymphangiogenesis as a means of dissemination of lymphatic metastases. Appropriate combination therapies may be used in the future to both prevent and treat metastatic disease through the rational use of antiangiogenic and antilymphangiogenic therapies in ways that are informed by the current and future work in the field.

Key Words: Angiogenesis, angiogenic, cancer, EMT, endothelial cell, lymphangiogenesis, malignant, metastasis, tumor, vessel

(Angiogenesis and Tumor Growth)

Angiogenesis and Tumor Growth

Normal epithelial cells multiply during development or embryogenesis, but adult epithelial cells divide more sparingly, such as in response to regular turnover and renewal or during tissue expansion and wound healing. Carcinoma cells originate from the transformation or mutation of normal epithelial cells. Their unrestricted cellular proliferation results in a mass of cells within the epithelial compartment either protruding out from the surface (i.e., in the epidermis), protruding into the lumen of the tube (e.g., in the colon), or filling the lumen of a gland (e.g., in the prostate). This stage is called "carcinoma in situ" or "intraepithelial neoplasia" and is considered a precancerous neoplasm. If found, these lesions can be surgically removed quite easily. For example, polyps are routinely removed during colonoscopy examinations. Such benign tumors are localized with no chance of spreading because the epithelial layer is not vascularized. These confined tumors receive their oxygen and nutrients via diffusion from capillaries that lie under the basement membrane of the epithelial layer. Eventually, equilibrium may be reached between tumor cell proliferation and apoptosis such that there is no net gain, and the overall size of the mass does not increase. Visualization of these dormant yet viable lesions is more difficult in other tissues. For example, prostatic intraepithelial neoplasia is found in approximately 16% of all men who undergo prostate biopsies each year (2015 Annual Report on Prostate Disease, Harvard). A diagnosis of prostatic intraepithelial neoplasia presents a major dilemma because such tumors may remain dormant for years, whereas others will progress.

In vitro, tumor cells grown in spheroids have an upper size limit based on the distance that nutrients can diffuse in the media into the core of the spheroid. Similarly, tumors grown in organs ex vivo can expand to only ~1 to 2 mm³. In vivo, tumors cannot expand beyond the diffusion limit of nutrients from the nearest capillary, which is approximately 100 to 500 μm, unless new blood vessels grow toward the tumor. The process by which these tumor-associated neovessels sprout from existing blood vessels was first referred to as "tumor angiogenesis" by Dr Judah O'Reilly in 1971. In his seminal article in The New England Journal of Medicine in 1971, he hypothesized that "endothelial cells (ECs) may limit tumor expansion" and that "for every increase in tumor diameter there must be an increase in tumor vascularization." Folkmann proposed that the mitotic index of the tumor cells and the ECs in the capillary were interdependent with a symbiotic relationship as an ecosystem. In fact, many years before they were first purified, he hypothesized that tumors must secrete what he called "tumor angiogenesis factor." (Cancer J 2015;21: 267–273)

We now appreciate that angiogenesis is a normal physiological process involving the proliferation, migration, and morphogenesis of ECs from existing vessels into new blood vessels. Angiogenesis is an active process during development and in physiological processes such as wound healing or thickening of the endometrium during the menstrual cycle. It is distinguished from vasculogenesis, which is the de novo formation of the first vessels from angioblasts in an embryo. From the point of view of the ECs, tumor angiogenesis and normal angiogenesis are quite similar. They differ mainly in the source of the EC mitogen or chemoattractant. Notably, tumor neovascularization differs in tumors originating in nonvascularized epithelium (e.g., in transgenic mice overexpressing a tissue-specific oncogene) versus those in the vascularized dermis or lamina propria (e.g., tumor cells injected or implanted as a xenograft). The former requires an initial invasion of the epithelial basement membrane to gain access to underlying blood vessels, called the vertical growth phase. A second difference is that normal angiogenesis is time-limited, whereas tumor angiogenesis continues as long as the tumor is in place.

As tumor expansion occurs, the inner tumor cells get further from their blood supply and become relatively hypoxic. Hypoxia up-regulates the expression of many angiogenic growth factors in tumor cells. (For a list of angiogenesis stimulators, see the reviews by Klagsbrun and D’Amore, Folkmann, Iruela-Arispe and Dvorak, and Fidler et al) Briefly, the process of tumor angiogenesis follows these sequential steps: (1) tumor cells release growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), to attract ECs toward the tumor mass; (2) ECs (and other cells) secrete enzymes to degrade the proteins in the basement membrane of the capillary or postcapillary venule (never arteries); (3) ECs (tip cells) begin to migrate or sprout toward the source of the stimulant, usually at right angles to the existing vessel; (4) ECs continue to migrate, and cells behind the leading tip cell, called stalk cells, proliferate and align in a single-file orientation; (5) the aligned ECs then morph and create a lumen or tube in the center of the newly formed vessel; (6) blood is perfused into the lumen of the new...
sprout. New capillaries typically loop and interconnect to create a plexus within the tumor. This process is illustrated in Figure 1A, B.

Tumor-associated capillaries are notoriously abnormal. A detailed review of their pattern and structure is outlined by Dvorak and colleagues. Briefly, tumor vessels are tortuous and mis-guided. They are malformed and hyperplastic. Because of the high expression of VEGF (and other factors) in the tumor environment, tumor vessels are also highly permeable and leaky. This leads to a high volume of fluid within the tumor microenvironment and high interstitial fluid pressures. Normal capillaries are stabilized by intertinent smooth muscle cells called pericytes that surround the capillary abluminally to support its structure and patency and to promote its survival and function. In contrast, tumor vessels are immature, show rapid turnover, and generally lack sufficient pericyte coverage.

The initiation of tumor angiogenesis is a pivotal point in tumor progression and has been called the “angiogenic switch.” This hallmark of cancer denotes the shift from dormancy to progressive growth. Importantly, both benign neoplasms (such as a uterine fibroid cyst or vascular malformation) and malignant cancers gain angiogenic potential as they grow in size and acquire additional vascularization. Therefore, the angiogenic switch is often mistaken as a sign of malignancy. Rather, angiogenesis should be viewed as an “organizing principle” that is required to obtain any significant gain in tissue mass in a variety of diseases.

Many cells within the tumor microenvironment, in addition to tumor cells, can secrete angiogenic factors that cause increased vascularization. Most notable are tumor-associated fibroblasts and macrophages. Tumor cells mixed with fibroblasts isolated from human breast carcinomas, called carcinoma-associated fibroblasts, can dramatically increase tumor angiogenesis and tumor growth rates. Specifically, carcinoma-associated fibroblasts secrete SDF1, which promotes neovessel formation and endothelial progenitor cell recruitment. Tumor-associated macrophages deliver MMP9 to the tumor microenvironment, and MMP9 cleaves VEGF from matrix sequestration—freeing it to stimulate vessel growth, motility, and permeability. It is worth mentioning that many growth factors are multipotent within the tumor environment. For instance, epidermal growth factor (EGF) is a robust stimulator of tumor cells and ECs. Tumor vessels up-regulate EGF receptor, unlike normal vessels, and therefore respond to stimulation via EGF.

ANGIOGENESIS AND METASTASIS

Primary carcinomas rarely cause patient death (with the exception of lung and liver cancer). Rather, most carcinoma-related mortality is due to complications associated with metastasis. The term metastasis was first contrived by Joseph-Claude-Anthelme Récamier (reviewed by Talmadge and Fidler). Metastasis refers to the transfer of cancer cells from one part of the body to another. The spread of cancer cells from organ to organ is incredibly inefficient; only 0.01% of cells that leave the primary site go on to create a distant metastasis. Why, then, do so many cancer patients succumb to metastatic disease? Because tumors are composed of billions of cancer cells, and 1 to 4 million cancer cells can be shed into the circulation each day from a 1-g tumor. In addition, hundreds of metastatic colonies can arise in an individual organ, and the unrestricted growth of these colonies can lead to replacement of normal tissue by tumor in essential organs, such as the lung, liver, brain, and bone marrow.

Unlike carcinomas and melanomas, sarcomas rarely metastasize. Likewise, small avascular dormant lesions do not metastasize. In general, metastatic colonization is a late step in the progression of carcinoma; therefore, small tumors found in the early stages can be cured by surgical excision. The metastatic process is described as a cascade of interrelated and sequential events that are each rate-limiting (reviewed by Fidler et al, Talmadge and Fidler, Valastyan and Weinberg). The ordered steps of metastasis are similar for most malignant neoplasms, yet the route and destination may vary:

1. Angiogenesis, depicted in Figure 1B. Cancer cells must develop a vascular (or lymphatic) network. Neovessels provide not only nutrients for the tumor to grow but also an escape route for the tumor cells to enter the circulation. In general, the larger the tumor and the more vascular density within the tumor, the greater the chance a tumor cell will escape.
2. Intravasation. Malignant tumor cells must detach from neighboring cells and matrix, invade through the capillary basement membrane, and migrate through the endothelial barrier to enter the bloodstream. The transition to the mesenchymal phenotype is thought to aid in this migratory process. Alternately, detached tumor cells may be swept up in the mixture of fluid and cells that flow into lymphatic capillaries (described in more detail below). All lymphatic fluid is eventually recycled into the venous system, so either route may result in tumor cells in the circulatory system, depicted in Figure 1C, D.
3. Survival in circulation. The shear stress exerted by the pulsatile flow of blood and high serum concentration is an inhospitable environment for tumor cells. Most circulating tumor

FIGURE 1. Illustration of steps in the metastasis process. A, Early carcinomas are confined to the epithelial compartment and receive their oxygen and nutrients by diffusion. B, To grow beyond 1 mm³, tumors acquire neovascularization. Increased tumor-associated vascular and lymphatic density increases the propensity for tumor dissemination. Blood vessel, red; lymphatic vessel, green. C, Tumor cells can escape via lymphatic vessels and arrest in sentinel LNs. Tumor cells in the LN may invade local blood vessels or remain in the lymphatic system to be recycled to the vascular system. D, Tumor cells may also invade blood vessels in the tumor (intravasation), travel in the circulation, and exit in the new organ environment (extravasation). Tumor expansion again requires angiogenesis in the secondary site. Tumor cells can metastasize via the vascular system (B → D) or the lymphatic system (B → C → D).
cells (CTCs) will die in the first 24 hours by either attrition or direct cytotoxicity and lysis by natural killer cells on immuno-surveillance. Platelets protect tumor cells in the initial hours after intravasation, and platelet-derived transforming growth factor β may enhance tumor cell extravasation by inducing epithelial-to-mesenchymal transition (EMT) pathways.

(4) Extravasation. Tumor cells are thought to transmigrate through interendothelial junctions to extravasate in distant organs. Recent studies show that lymphocyte (and possibly tumor cell) diapedesis can also occur through transcellular routes. Although tumor cells extravasate via venules, they often then migrate to arterioles where the oxygenation state of the tissue is higher. Cancer cells do not always lodge in the first vascular bed they encounter; rather, tumor cell-host interactions dictate metastatic patterning.

(5) Secondary tumor formation (depicted in Figure 1D). Extravasation does not necessarily result in a successful metastasis. Most single cancer cells that get out of the circulation either apoptosis, get eliminated by immune cells, or just remain dormant. To eventually form macrometastases, malignant tumor cells must proliferate and again undergo angiogenesis to result in a clinically relevant secondary tumor. Metastatic tumors can remetastasize to tertiary sites and can even reimplant in the primary tumor.

One of the strongest lines of evidence linking angiogenesis and metastasis is that tumor microvessel density (MVD) correlates with increased metastatic potential and poor survival in nearly all forms of malignancy (discussed in detail below). Angiogenesis is essential for the growth of lung micrometastases. Some studies suggest that bone marrow-derived endothelial progenitor cells contribute to the early angiogenic stages of metastatic growth, whereas others propose that cooption of normal vessels is a mechanism for metastasis vascularization. Antiangiogenic therapies in preclinical trials may reduce both the incidence and severity of metastasis (see Antiangiogenesis Therapy and Metastasis).

Somewhat paradoxically, Folkman and colleagues showed that the surgical excision of a murine primary tumor could stimulate the growth of dormant metastases. The mechanism for this angiogenic switch in the metastatic site was due to the loss of an endogenous angiogenesis inhibitor, angiostatin, being secreted systemically by the primary tumor. Similarly, the knockout of other endogenous angiogenesis suppressors promotes tumor vascularization and metastasis. These data further strengthen the link between angiogenesis and metastasis.

Interestingly, pericytes also play a role in metastasis. Pericytes help to control the patency of capillary lumens and are therefore essential for tumor cell dissemination. MMP9 knockout mice lack pericytes and have collapsed morphology in vessels and diminished metastasis.

Besides extravasation, CTCs may also lodge in the microvessels of distant organs and begin to grow intraluminally. These cells could eventually rupture the vessel wall and gain access to the underlying tissue. Circulating tumor cells also represent “metastatic intermediates,” as they have accomplished initial steps of the metastatic process. As such, these cells are now highly valued as surrogate markers of therapeutic efficacy in preclinical and clinical trials. In other words, if a drug is effective at shrinking a tumor, then fewer CTCs should be released into the circulation. High CTC numbers correlate with poor clinical outcome, and drugs that reduce CTC levels may be candidates for clinical development.

The seed and soil hypothesis, postulated by Paget and popularized by Fidler, Fidler and Poste, and Fidler et al., describes the tropism of certain tumors for specific organs. This may be due to the fact that certain tumors have receptors for specific growth factors preferentially secreted from a particular organ. For instance, colon cancer cells expressing cMET receptor homed to the liver, which secretes hepatocyte growth factor. Alternatively, tissue-specific vascular beds may have “zip codes” or surface markers recognized by tumor cells. In fact, certain primary tumors are found to prepare a premetastatic niche in the secondary location prior to tumor cell arrival. Conversely, primary tumor cells secreting prosaposin inhibited metastatic colonization by systemically inducing the expression of thrombospondin 1 in lung stromal cells.

**LYMPHANGIOGENESIS AND METASTASIS**

Lymphatic vessels originally sprout from veins in mid-gestation (E9-9.5 in mice). However, in the adult, the lymphatic system is structurally and functionally quite different than the blood vascular system. The vascular system is a closed, circulatory system in which blood is pumped through arteries, arterioles, capillaries, venules, and veins to deliver oxygen and nutrients to all of the cells in the body. Lymphatic vessels, on the other hand, comprise a unidirectional, fluid recycling system. Fluid (and cells) are taken up into lymphatic capillaries, and fluid propulsion is channeled down lymphatic collecting ducts (containing valves) toward lymph nodes (LN). Fluid may be filtered through several LNs, but ultimately all lymphatic fluid is emptied back into the venous system.

At the microvessel level, lymphatic capillaries are structurally dissimilar to vascular capillaries. Lymphatic capillaries are lined with a thin endothelium that lacks or has minimal basement membrane and lacks pericyte coverage. Anchoring filaments function to open small gaps at button junctions between neighboring lymphatic ECs (LECs) when interstitial fluid pressure rises. These intercellular LEC gaps are essential to regulate fluid dynamics and to prevent tissue edema by increasing drainage. They also allow the infiltration of immune cells, such as dendritic and Langerhans cells, to gain access to the LN. Most notably, these openings offer direct and passive access of tumor cells to the lymphatic system. The LN has a filter function that initially protects the host but may act as a breeding ground or hub for tumor cell pooling that eventually spreads to other sites. Entrance to the lymphatic system may not guarantee that tumor cells will reach the LN, as some tumors were required to have the “key” (expression of CCR8) in order to unlock the “gate” (CCL1 expression by LEC) at the LN sinus.

Malignant carcinomas of virtually every type are reported to metastasize to regional LNs. Some types of cancer spread via the vascular and lymphatic systems simultaneously, whereas others seem to metastasize in a metachronous manner with a tumor in the sentinel (draining) LN preceding those metastases in distant organs. In these sequential cases, lymphadenectomy can be curative. The TNM staging system used for tumor classification is based on the assumption that most tumors progress sequentially from a primary tumor to LN metastasis and then to distant metastasis. Tumor-associated LECs can secrete chemotactic factors to attract malignant tumor cells. The process of tumor lymphangiogenesis is similar to that of tumor angiogenesis in that it is growth factor mediated, results in ill-formed hyperplastic capillaries, and increases the microvascular density and area through which tumor cells could escape. Yet, tumor lymphangiogenesis differs from angiogenesis in that mainly the peritumoral lymphatic vessels are enlarged, and few lymphatic capillaries sprout inside the tumor. In histological human melanoma patient samples, the
parameter of the lymphatic vessel area was more predictive of metastasis than the criterion standard, tumor thickness. Tumors engineered to overexpress the lymphangiogenic factor, VEGF-C, stimulated tumor-associated lymphangiogenesis inside and around the tumor periphery and increased LN metastasis. Conversely, silencing VEGF-C in another model ablated only the intratumoral LECs, and LN metastasis was unchanged, suggesting that peritumoral lymphatic vessels are sufficient for LN metastasis.

Once tumor cells are in the LN, they essentially become a secondary tumor and can either invade blood capillaries in the LN or keep traveling in the lymph to eventually enter the circulation. The lymphatic system is now recognized as a significant source of CTCs in the blood. Lymph node metastasis is correlated with an increased risk of distant metastasis and a poor clinical outcome in most cancers. An unresolved question is the extent to which LN metastases contribute directly to distant metastases in vital organs.

**VASCULAR DENSITY AND METASTASIS**

Angiogenesis is a hallmark of cancer and is linked to metastasis. Therefore, the ability to accurately and reliably quantify the vasculature within a tumor is essential. In fact, Folkman discussed tumor capillary density in his first angiogenesis article and ranked brain tumors as the most highly vascular, followed by carcinomas, sarcomas, and chondrosarcomas, in that order. Later, he and Dr. Noel Weidner et al. published the methodologies for obtaining and calculating the MVD within tumors. Typically, MVD is calculated as the number of vessels stained positive by immunostaining for specific EC markers per high-power microscope field (200× or 0.74 mm²). Because it is not feasible to count every field, Weidner et al. popularized the notion of counting “hot spots” or areas of high vascular density. As MVD is heterogeneous throughout the tumor but tends to increase toward the periphery of the tumor, choosing the best field to count is a topic of debate. In addition, which marker of tumor blood vessels to choose and which antibodies to use for staining are not standardized, with popular options being CD31, CD34, and von Willebrand factor.

Regardless of the technical details, MVD is a reproducible prognostic indicator of metastasis for certain cancers. A meta-analysis combining the data from 87 articles regarding MVD in lymph node metastasis for certain cancers. A meta-analysis combining the data from 87 articles regarding MVD in tumors demonstrated that the MVD in LN or keep traveling in the lymph to eventually enter the circulation. The lymphatic system is now recognized as a significant source of CTCs in the blood. Lymph node metastasis is correlated with an increased risk of distant metastasis and a poor clinical outcome in most cancers. An unresolved question is the extent to which LN metastases contribute directly to distant metastases in vital organs.

Newer techniques, especially for the assessment of anti-angiogenic therapeutic efficacy, have used proliferative capillary index (CD31/Ki67 vessels) or microvesSEL pericyte coverage index to measure vessel maturity. In addition, lymphatic vascular density, lymphatic vessel infiltration, vessel caliber using magnetic resonance imaging, fluorescence-activated cell sorting analysis, and perfusion with fluorescent lectins have been used to accurately calculate and predict vascular phenotypes. The use of automated counting programs and new software has greatly enhanced the standardization of these methods.

**ANTIANGIOGENESIS THERAPY AND METASTASIS**

If angiogenesis is a critical and rate-limiting step in tumor progression, then it follows that blocking angiogenesis should inhibit cancer progression. Indeed, Folkman postulated in 1971 that drugs could be used to inhibit angiogenesis years before the first angiogenesis inhibitor was found. In 1980, interferon αβ was shown to inhibit capillary EC motility and therefore represented the first experimental evidence for angiogenesis inhibition. Now the list of angiogenesis inhibitors is too numerous to list here individually but includes classes of molecules such as endogenous proteins (e.g., thrombospondin), soluble receptors, receptor tyrosine kinase inhibitors (small molecule inhibitors), siRNA to angiogenic factors, and antibodies to individual growth factors and receptors. It is important to mention that malignant tumors secrete multiple angiogenic growth factors at the same time. Vascular endothelial growth factor has been the most well-studied angiogenic factor. It is apparent that the majority of angiogenesis inhibitors currently in clinical trials target the VEGF signaling pathway. Many tumors respond initially to VEGF-targeted therapies only to relapse because of tumor evasion. Tumor evasion here refers to a tumor cell up-regulating additional angiogenic factors in response to one pathway being blocked and is caused by the redundancy of angiogenic factors produced by tumor cells. For example, tumors exposed to anti-VEGF strategies may up-regulate FGF or other angiogenic factors. Combination therapies are now being explored. Evasion from antiangiogenesis drugs is often misinterpreted as EC resistance. In the case of anti-VEGF drugs, it is actually the tumor cells that are the target because the tumor cell secretes the VEGF. Tumor cells exposed to a hypoxic and acidic environment, because of the pruning of blood vessels during antiangiogenic therapy, may respond to the stress in a multitude of ways, such as by switching to anaerobic metabolism, undergoing EMT, or altering its secreted factors, which affect inflammation and fibrosis. Angiogenesis inhibitors have been divided into two main classes depending on their mode of action and their target cell. Direct angiogenesis inhibitors act directly on the ECs, but may have...
off-target effects on tumor cells as well. These inhibitors include endostatin, angiostatin, thalidomide, TNP470, and semaphorin 3F. Direct antiangiogenesis inhibitors typically block EC proliferation or motility regardless of the stimulator. Their mode of action may be downstream of the growth factor-induced signal transduction cascade, and therefore incubation of ECs with these direct inhibitors trumps the action of growth factors such as VEGF or FGF. Evasion or resistance is less likely to occur from direct antiangiogenesis inhibitors. Alternately, indirect antiangiogenesis inhibitors block growth factors or pathways of cells other than ECs. Examples of indirect inhibitors include antibodies to growth factors such as anti-VEGF, soluble receptors of VEGF receptor, or inhibitors of pericyte recruitment. Because indirect antiangiogenesis inhibitors block factors emanating from tumor cells, they are likely to result in resistant tumors that produce alternative angiogenic factors.

Chemotherapies are cytotoxic drugs that inhibit the growth of any rapidly dividing cell, which in a cancer patient means the tumor cell. These antimitotic drugs are typically given at maximum tolerated doses and are very toxic to the host cells as well as the tumor cells. Therefore, to prevent major bone marrow suppression, the patient is given the drug for a set period and then given a break or “holiday” from the drug to recover their bone marrow cells. When given at low doses on a continuous schedule, chemotherapy can target the ECs and not the tumor cell. Antiangiogenic chemotherapy was successful at inhibiting the expansion of multiple tumor types in vivo. This type of regularly scheduled or continually dosed chemotherapy is also called “metronomic chemotherapy.”

In 2009, two independent laboratories reported that antiangiogenic therapies stimulated metastasis. More recently, a similar response was found with the same drug in liver cancer. Overinterpretation of these results should be cautioned. One of the drugs used was sunitinib, which is a receptor tyrosine kinase inhibitor of the VEGF receptor and PDGF receptor that targets multiple cell types in the tumor microenvironment in addition to tumor-associated ECs and pericytes. Sunitinib was given at a high dose of 120 mg/kg. The experiment was designed such that the sunitinib was given prior to tumor injection as a “conditioning.” Under these circumstances, there is no tumor at the time of drug delivery; therefore, there is no angiogenesis to inhibit. Rather, the drug was actually used to treat normal ECs in the lung, which may then be called antivascular therapy. Regardless, when tumor cells were injected intravenously into the mouse pretreated with sunitinib, greater numbers of tumor cells were able to colonize the lungs and establish experimental metastases. One interpretation of these results is that high doses of a kinase inhibitor of the VEGF pathway in normal mice may result in the blocking of VEGF survival signals and subsequent EC dropout. The “damaged” lung vessels may have been more easily traversed by the tumor cells, resulting in lung colonization. Increased metastasis was not found when sunitinib was given at later time points. These experimental conditions are unlikely to occur in the clinic, as patients are not given this drug prophylactically.

FUTURE DIRECTIONS

Cancer therapy, as used today, is most commonly used to treat metastatic disease and not primary tumors. Little work has been done to distinguish the differences between the causes, types, and patterns of angiogenesis in secondary versus primary tumors. It is critical that these studies be undertaken, where possible, in patient-derived samples or in genetically engineered mouse models of metastatic cancers. Similarly, preclinical studies of antiangiogenesis therapy and evasion should always be conducted in animal models where established metastases are present. Until such studies are the norm, we are unlikely to develop angiogenesis inhibitors that are highly effective in the metastatic setting.

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