Regulation of Neoplastic Angiogenesis

Isaiah J. Fidler

The progressive growth of neoplasms and the production of metastasis depend on the development of adequate vasculature, i.e., angiogenesis. The extent of angiogenesis is determined by the balance between positive- and negative-regulating molecules that are released by tumor and host cells in the microenvironment. The growth of many neoplasms is associated with the absence of the endogenous inhibitor of angiogenesis, interferon beta (IFN β). A survey of multiple mouse and human tumors shows a lack of IFN β associated with extensive angiogenesis. Therapy with IFN α or β either by subcutaneous injection of the protein or by introduction of viral vectors that contain the IFN β gene inhibit angiogenesis and, hence, progressive tumor growth. [J Natl Cancer Inst Monogr 2000;28:10–4]

Cancer Metastasis

The major cause of death from cancer is metastases that are resistant to conventional therapy. One major obstacle to the treatment of metastasis is the biologic heterogeneity of neoplasms *(1).* A second obstacle is the ability of different organ environments to modify a metastatic tumor cell's response to therapy *(2,3).* A better understanding of the mechanisms that regulate the process by which tumor cells invade local tissues and spread to distant organs should lead to the design of more effective therapy.

The process of cancer metastasis consists of a series of sequential steps, each of which can be rate limiting *(1).* After the initial transforming event, growth of neoplastic cells must be progressive. Extensive vascularization must occur if a tumor mass is to exceed 1 mm in diameter *(4).* The next step is local invasion of the host stroma that occurs by several mechanisms *(5).* Small tumor cell aggregates then detach and embolize next and some tumor cells that survive the trauma of the circulatory system arrest in the capillary beds of organs extravasate into the organ parenchyma, proliferate, and induce angiogenesis to allow expansion of the lesion *(1).*

The outcome of metastasis depends on the interactions of tumor cells with various host factors *(1,6,7).* The pattern of metastasis is not random but rather is determined by factors that are independent of vascular anatomy, rate of blood flow, and the number of tumor cells delivered to each organ *(1).* The search for factors that regulate metastasis began in 1889 when Paget analyzed postmortem data of women who died of cancer and noticed the high frequency of metastasis to the ovaries and the different incidence of skeletal metastases associated with different primary tumors. Paget concluded that the organ distribution of metastases is not a matter of chance and suggested that metastases develop only when the "seed" (certain tumor cells with metastatic ability) and the "soil" (colonized organs providing growth advantage to the seeds) are compatible *(8).* In recent years, Paget's hypothesis has received considerable experimental and clinical support *(1,9–11).* Site-specific metastasis has been demonstrated with many transplantable tumors and has also been documented in autochthonous human tumors in patients with peritoneovenous shunts *(12,13).*

A current definition of the "seed and soil" hypothesis encompasses three principles. First, neoplasms are biologically heterogeneous *(1,14).* Second, the process of metastasis is highly selective, favoring the survival and growth of a small subpopulation of cells that pre-exist in the heterogeneous parent neoplasm *(6).* Third, the outcome of metastasis depends on multiple interactions of metastatic cells (seed) with homeostatic mechanisms (soil) *(2).* The majority of malignant neoplasms actually usurp homeostatic mechanisms to gain growth advantage *(1,6,7).* Neoplastic angiogenesis is an excellent example.

Tumor Angiogenesis

The survival and growth of cells depend on an adequate supply of oxygen and nutrients and on the removal of toxic molecules. Oxygen can diffuse from capillaries for only 150–200 mm. When distances of cells from a blood supply exceed this, cell death follows *(15).* Thus, the expansion of tumor masses beyond 1 mm in diameter depends on neovascularization, i.e., angiogenesis *(4,16).* The formation of new vasculature consists of multiple, interdependent steps. It begins with local degradation of the basement membrane surrounding capillaries, followed by invasion of the surrounding stroma and migration of endothelial cells in the direction of the angiogenic stimulus. Proliferation of endothelial cells occurs at the leading edge of the migrating column and the endothelial cells begin to organize into three-dimensional structures to form new capillary tubes *(4,17).* Differences in cellular composition, vascular permeability, blood vessel stability, and growth regulation distinguish vessels in neoplasms from those in normal tissue *(18).*

The onset of angiogenesis involves a change in the local equilibrium between proangiogenic and antiangiogenic molecules *(19).* The major proangiogenic molecules include fibroblast growth factor (FGF) family members, vascular endothelial cell growth factor or vascular permeability factor (VEGF/VPF), interleukin 8 (IL-8), angiogenin, platelet-derived endothelial cell growth factor, platelet-derived growth factor, and matrix metalloproteinases *(4,20,21).* Many different proangiogenic or antiangiogenic molecules are present in different tissues *(4,22).* In normal tissues, factors that inhibit angiogenesis predominate (e.g., interferon beta [IFN β], tissue inhibitor of metalloproteinases) *(4,23),* whereas, in rapidly dividing tissues, factors that stimulate angiogenesis predominate. Our laboratory has investigated the role of cell density in the regulation of bFGF expression in human renal cell carcinoma cells or human endothelial cells. Dividing cells expressed higher levels of bFGF (both at

Correspondence to: Isaiah J. Fidler, D.V.M., Ph.D., Department of Cancer Biology, Box 173, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030 (e-mail: ifidler@notes.mdacc.tmc.edu).

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messenger RNA [mRNA] and protein levels) than nondividing cells *(24).* In contrast, nondividing cells express higher levels of VEGF/VPF than dividing cells *(25).*

Regulation of Angiogenesis by the Microenvironment

The production of bFGF and IL-8 by tumor or host cells or the release of angiogenic molecules from the extracellular matrix induces the growth of endothelial cells and the formation of blood vessels. Data from our laboratory have demonstrated that the organ microenvironment can directly contribute to the induction and maintenance of the angiogenic factors bFGF *(26,27)* and IL-8 *(28).* For example, in patients with renal cell carcinoma, the level of bFGF in the serum or urine inversely associated with survival *(29,30).* Human renal cancer cells implanted into different organs of nude mice had different metastatic potentials: Those implanted into the kidney produced a high incidence of lung metastasis, whereas those implanted subcutaneously were not metastatic *(26).* Histopathologic examination of the tumors revealed that subcutaneous tumors had few blood vessels, whereas the tumors in the kidney had many *(26).* The subcutaneous (or intramuscular) tumors had a lower level of mRNA transcripts for bFGF than did continuously cultured cells, whereas tumors in the kidney of nude mice had 20-fold the levels of bFGF mRNA and protein level *(26,27).*

Constitutive expression of IL-8 directly associates with the metastatic potential of the human melanoma cells *(28).* IL-8 contributes to angiogenesis by inducing proliferation, migration, and invasion of endothelial cells *(31).* Several organ-derived cytokines (produced by inflammatory cells) can increase expression of IL-8 in normal and tumorigenic cells *(32).* IL-8 expression was increased in co-culture of melanoma cells with keratinocytes (skin), whereas it was inhibited in cells co-cultured with hepatocytes (liver). Similar results obtained with conditioned media from keratinocyte and hepatocyte cultures suggested that organ-derived factors, e.g., IL-1 and transforming growth factor-β, can modulate the expression of IL-8 in human melanoma cells *(32).*

The influence of the microenvironment on the expression of VEGF/VPF, angiogenesis, tumor cell proliferation, and metastasis was investigated with the use of human gastric cancer cells implanted in orthotopic (stomach) and ectopic (subcutaneous) sites in nude mice. Tumors in the stomach were highly vascularized and expressed higher levels of VEGF/VPF than did subcutaneous tumors *(33).* Moreover, only tumors implanted in the stomach produced metastasis, suggesting that the expression of VEGF/VPF vascularization and metastasis of human gastric cancer cells are regulated by the organ microenvironment.

Molecular Determinants of Angiogenesis in Cutaneous Hemangiomas

Infantile cutaneous hemangiomas represent a unique form of pathologic angiogenesis in which endothelial cell tumors grow rapidly in the first year of life (proliferative phase), followed by a slow regression during the next 5 years (involuting phase) and eventual involution or complete regression (involuted phase) by the age of 10–15 years *(34).* Long-term daily systemic treatment with IFN α has been shown to accelerate the involution of fatal hemangiomas *(34–39).* To determine whether the progression and involution of infantile cutaneous hemangiomas were associated with overexpression of proangiogenic molecules or the lack of antiangiogenic molecules, a large number of hemangioma specimens by immunohistochemistry was analyzed. The results showed that proliferating hemangiomas expressed bFGF and VEGF/VGF but not IFN β (mRNA and protein) *(40).* A surprising finding was that the epidermis directly overlying proliferating hemangiomas was hyperplastic, whereas the epidermis overlying involuted hemangiomas or the epidermis from an unaffected site was not *(40).* The hyperplastic epidermis expressed bFGF, VEGF/VPF, and IL-8 but not IFN β, whereas the normal epidermis expressed both positive- and negative-angiogenic molecules *(40).* These data raised the possibility that the proliferating hemangiomas induced hyperplasia in the surrounding normal tissues (epidermis), leading to production of bFGF and VEGF/VPF but not IFN β *(40),* supporting the concept that neoplastic cells subvert and usurp host homeostatic mechanisms for their growth advantage *(1,2).*

To study the relationship between hemangiomas and the microenvironment, an *in vivo* model was developed for epidermal hyperplasia and angiogenesis, using UVB irradiation of mice *(41).* Mice exposed to 10 kJ/m^2 UVB developed epidermal hyperplasia accompanied by angiogenesis and telangiectasia during the first week after irradiation, but these conditions slowly subsided over the following weeks. The first striking event after UVB irradiation was the increase in production of bFGF in the keratinocytes of the epidermis *(41).* The increase in bFGF preceded or at least coincided with the division of epidermal cells recognized by immunohistochemical staining with antibodies to proliferating cell nuclear antigen. Marked hyperplasia and angiogenesis followed immediately. The expression of VEGF/VPF was slightly increased by day 5. Of interest, the expression of IFN β in the epithelium decreased with epidermal hyperplasia but was re-expressed as the hyperplasia and angiogenesis subsided *(42).*

Systemic therapy with the use of recombinant IFNs produces antiangiogenic effects in vascular tumors, including hemangioma *(34–39),* Kaposi's sarcoma *(43–46),* melanoma *(47),* basal cell and squamous cell carcinomas *(48),* and bladder carcinoma *(49).* These tumors have also been documented as producing the high levels of bFGF often detectable in the urine or serum of these patients *(29,30,50).* These findings, along with our *in vivo* observations, prompted us to investigate whether IFNs could modulate the expression of the angiogenic molecule bFGF. We found that IFN α and IFN β but not IFN γ decreased the expression of bFGF mRNA and protein in human renal cell cancer (HRCC) as well as in human bladder, prostate, colon, and breast carcinoma cells *(51).* The inhibitory effect of IFN α and β on bFGF expression was cell-density dependent and independent of the antiproliferative effects of IFNs *(51,52).* We also confirmed that IFN can inhibit bFGF production in an *in vivo* model system. Systemic administration of human IFN α decreased the *in vivo* expression of bFGF, decreased blood vessel density, and inhibited tumor growth of a human bladder carcinoma implanted orthotopically in nude mice *(53).*

Antiangiogenic Activity of IFN β

The IFN family consists of three major glycoproteins that exhibit species specificity: leukocyte-derived IFN α, fibroblast-derived IFN β, and immune cell-produced IFN γ. Although IFN α and IFN β share a common receptor (the type I IFN receptor) and induce a similar pattern of cellular responses, cer-

tain cellular reactions can be stimulated only by IFN β, probably by the phosphorylation of a receptor-associated protein that is uniquely responsive to IFN β *(54).* In addition to their well-recognized activity as antiviral agents, IFNs regulate multiple biologic activities, such as cell growth *(55,56),* differentiation *(57),* oncogene expression *(58,59),* host immunity *(60–62),* and tumorigenicity *(63–68).* IFNs can also inhibit a number of steps in the angiogenic process. IFN has antiproliferative properties, especially on tumor cells *(69–71),* an effect that has also been demonstrated on endothelial cells *in vitro.* IFN α can inhibit FGF-induced endothelial proliferation *(72),* and IFN γ can inhibit endothelial proliferation *(73).* IFN α and IFN γ have been shown to be cytostatic to human dermal microvascular endothelial cells *(74)* and to human capillary endothelial cells *(75).*

The antiangiogenic effect of IFNs cannot be explained solely on the basis of inhibition of endothelial cell proliferation. For example, IFN α/β can also inhibit the endothelial cell migration step of angiogenesis *(76,77).* Subcutaneous injection of IFN α/β adjacent to a wound delayed the healing process by inhibiting the proliferation, migration, and invasion of capillary buds, fibroblasts, and epithelium *(78,79).* IFN α/β injected intratumorally or peritumorally into tumor cells resistant to the antiproliferative effects of IFN damages blood vessels, leading to ischemia and necrosis *(80).* Moreover, we reported that IFN α/β can affect the expression of several angiogenic factors, including bFGF *(52,53),* IL-8 *(81),* and collagenase type IV *(82,83).*

Our laboratory recently demonstrated that IFN β gene therapy can eradicate tumor cells of various histologic origins and found that the sustained local production of murine IFN β could inhibit the tumorigenicity and metastasis of human and murine tumor cells implanted into nude mice *(84,85).* All human tumor cell lines transfected with the murine IFN β gene grew well *in vitro,* but none grew *in vivo.* IFN β-transfected cells prevented the outgrowth of parental or control-transfected cells when injected at the same site but not when injected at distant sites, suggesting that IFN β promoted a local lysis of the bystander cells *(84,85).* Similar results were found when human prostate cancer cells were infected with the murine IFN β gene with the use of a retroviral vector. Of interest, the transduced cells did not grow in nude mice when injected into the prostate. The regression of the tumors was directly associated with infiltration by macrophages and activation of inducible nitric oxide synthase *(86).* All transfected and transduced cells stimulated a high level of nitric oxide in murine macrophages, which associated with the vigorous antitumor activities. Therefore, the local production of IFN β can suppress tumorigenicity and metastasis, in part because of the activation of host effector mechanisms.

Conclusions

The angiogenesis within and surrounding neoplasms is due to an imbalance between proangiogenic molecules, e.g., bFGF, VEGF/VPF, IL-8, and antiangiogenic molecules (e.g., IFN). Tumor cells, normal host cells, and leukocytes all contribute to angiogenesis. The absence of IFN β from tumor beds is associated with robust angiogenesis. Restoring the balance between proangiogenic and antiangiogenic molecules provides an approach to the control of angiogenesis in neoplasms. Frequent systemic administrations of low-dose IFN α or β or the introduction of the IFN β gene to the tumor bed show great therapeutic promise in several animal models. Clinical trials should determine whether this approach is useful for therapy of human neoplasms.

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NOTES

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