Fundamental Concepts of the Angiogenic Process

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Abstract: The process of angiogenesis encompasses the growth and regression of capillary blood vessels. Angiogenesis is finely regulated at the molecular and genetic levels, not unlike other physiologic processes such as coagulation, glucose metabolism, and blood pressure. During the development of the field of angiogenesis research over the past three decades, fundamental concepts have been introduced along the way in an attempt where possible, to unify new data from a variety of different laboratories. I have assembled here the major concepts which underlie the angiogenic process as we currently understand it. Many of these are now taken for granted, but this was not always the case, and I have tried to show how they were developed. My goal is to provide a conceptual framework for those basic scientists or clinicians who may enter this rapidly expanding field. Each concept discussed here is accompanied by a few key references as a guide to the pertinent literature.

TUMOR GROWTH IS ANGIOGENESIS-DEPENDENT

The idea that tumor growth is angiogenesis-dependent, and the corollary concept that anti-angiogenic therapy could be employed to treat cancer, was first proposed in 1971 [1]. It was based on previous experiments [2], in which murine tumors implanted into isolated perfused canine thyroid glands remained viable, but failed to expand beyond 1 to 2 millimeters diameter. The tiny tumors grew rapidly when transplanted to mice. The difference was that tumors in mice were highly neovascularized, but the tumors in the isolated organs had no blood vessels. The 1971 paper [1] also discussed recent experiments in which tumors grown in the anterior chamber of the rabbit eye beyond the reach of the vascular bed of the iris, remained less than 1 mm^3 in size, but became neovascularized and grew rapidly, after the tumor was implanted on the vascular bed of the iris. This phenomenon was subsequently reported in more detail [3].

Pharmacologic evidence in support of this concept came in the late 1980s and 1990s from inhibition of a wide variety of tumor types by angiogenesis inhibitors such as TNP-470 [4].

Genetic proof that tumors are angiogenesis-dependent was reported by Arbiser et al, who showed that endothelial cells transformed by the SV40 oncogene, became immortal and formed dormant tumors of microscopic size in immunodeficient mice. However, there was no further tumor growth until after the dormant tumors were subsequently transfected by the *ras* oncogene [5]. Chin and DePinho reported that in large melanomas growing under the control of a doxycyline-inducible *ras* oncogene, that down-regulation of *ras* expression caused massive apoptosis of endothelial cells in the tumor's vascular bed within 12 hours, followed by tumor necrosis a few days later. Necrotic tumors underwent complete regression [6]. This experiment demonstrated that it was necessary for an oncogene to be continuously present to maintain the tumor it had induced, and that the *ras* oncogene mediated stimulation of angiogenic endothelium. Watnick et al, reported that *ras* induction of tumor angiogenesis is mediated by down-regulation of thrombospondin which requires cooperation with *c-myc* [7]. The most compelling genetic proof that tumor growth is angiogenesis-dependent comes from experiments reported by Lyden et al, in which tumors are unable to become angiogenic when implanted into transgenic mice in which one allele of the Id1 gene and two alleles of the Id3 gene have been deleted [8]. Subsequent repletion of these mice by transplantation of bone marrow containing normal progenitor endothelial cells carrying Id +/+ and Id3 +/+ genes, permitted neovascularization of the tumors, followed by rapid tumor growth [9].

TUMOR DORMANCY CAN RESULT FROM BLOCKED ANGIOGENESIS

Dormant tumors can be defined by their inability to expand beyond a microscopic size. Stable tumors may be defined by their inability to expand beyond a macroscopic size. It was long assumed that tumor dormancy could only be explained by cell cycle arrest (i.e., a G_0 state), or by 'immune surveillance.' However, blocked angiogenesis has been reported as an additional mechanism of dormancy [3,10-12]. Tumor dormancy by blocked angiogenesis appears to be a more common phenomenon in human tumors. In fact, it is now possible to isolate non-angiogenic tumor cells from human tumors and implant them in immunodeficient mice. These cells form non-angiogenic, dormant tumors of microscopic

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size (< 0.5 mm). This phenomenon has been called "no take," because such dormant tumors are usually invisible until the skin is opened. Beneath the skin lie tiny white dormant tumors. Histology reveals proliferating tumor cells balanced by apoptotic tumor cells and few if any microvessels [12]. These tumors can remain dormant for months to more than a year. Some tumor types spontaneously switch to the angiogenic phenotype within a few months (equivalent to years in a human), while others never switch during the normal life of the mouse (Nava Almog, unpublished data).

NORMAL CELL GROWTH IS CELL SHAPE DEPENDENT

We first demonstrated in 1978 that DNA synthesis is suppressed by restriction of cell spreading and is permitted by cell spreading [13]. Vascular endothelial cells constrained from elongating or spreading were refractory to mitogens in serum and were subsequently shown to be refractory to specific mitogens such as bFGF or VEGF [14]. DNA synthesis and proliferation were prevented regardless of whether the endothelial cells were prevented from spreading by crowding from neighboring cells, or because endothelial cells were plated on a substratum of reduced adhesivity (e.g., tissue culture wells coated with polyhydroxy ethylmethacrylate [polyHEMA]. This phenomenon provides one explanation for the general non-responsiveness of endothelial cells in the normal vasculature to circulating endothelial mitogens or pro-angiogenic molecules. In fact, the growth of new endothelial vascular sprouts from a pre-existing venule is almost always preceded by vasodilation of microvasculature followed by degradation of the basement membrane. Both events permit endothelial cells to elongate and to spread prior to their formation of new capillary blood vessels stimulated by a tumor [15]. DNA synthesis appears in elongated endothelial cells aligned linearly in the central portion of a growing capillary sprout, but not in foreshortened crowded endothelial cells at the proximal end of the sprout. A fundamental difference between normal cells and neoplastic cells is that neoplastic cells proliferate independently of shape constraints.

TUMOR CELLS PRODUCE SPECIFIC ANGIOGENIC PROTEINS

Until the early 1970s it was widely assumed that tumors did not produce specific angiogenic proteins. The conventional wisdom was that tumor vasculature was an inflammatory reaction to dying or necrotic tumor cells. The first endothelial mitogen, bFGF was isolated from the pituitary by Gospodarowicz in 1974 [16] and was subsequently purified from a tumor by Shing *et al.* [17], and then sequenced by Esch *et al.* [18]. Maciag's lab purified aFGF [19]. Dvorak's lab purified VPF (vascular permeability factor) [20], and Ferrara cloned it as VEGF (vascular endothelial growth factor) [21]. Since then, at least 14 pro-angiogenic proteins have been identified which are produced by tumor cells (TABLE 1) [22] and also [23].

Table 1. Positive regulators of angiogenesis most commonly produced by human tumors.

Most commonly produced by human tumors		
VEGF	45,000	Vascular endothelial growth factor
bFGF	18,000	Basic fibroblast growth factor
aFGF	16,400	Acidic fibroblast growth factor
PDGF	40,000	Platelet-derived growth factor
PD-ECGF	45,000	Platelet-derived endothelial growth factor
IL-8	40,000	Interleukin-8
HGF	92,000	Hepatocyte growth factor
EGF	6,000	Epidermal growth factor
Angiogenin	14,100	
Others		
TNF-alpha	17,000	Tumor necrosis factor alpha
TGF-beta	25,000	Transforming growth factor beta
TGF-alpha	5,500	Transforming growth factor alpha
Proliferin	35,000	
PLGF	25,000	Placental growth factor

One or more of these pro-angiogenic proteins may be over-expressed by tumor cells during the switch to the angiogenic phenotype [24]. Overexpression of pro-angiogenic proteins by tumor cells can be triggered by oncogenes, for example, increased expression of VEGF by *ras* [25], or by *bcl-2* [26] (TABLE 2). The fact that VEGF is the major or sole pro-angiogenic protein expressed by breast cancers in 60% of women at the time of first diagnosis, but that other breast cancers can express up to 6 different pro-angiogenic proteins [27], suggests that it may be prudent in the design of a clinical trial of an "indirect" angiogenesis inhibitor, to stratify patients so that the pro-angiogenic protein(s) produced by their tumor (as determined in a biopsy), is matched by an appropriate inhibitor. Indirect angiogenesis inhibitors (i.e, Avastin an antibody to VEGF), target a tumor cell oncogene, or its product, or the receptor for that product [22] [Fig. **1**]. In contrast, "direct" angiogenesis inhibitors (i.e., endostatin, tumstatin or angiostatin), target angiogenic endothelial cells in the tumor, and generally block the endothelial cell from responding (by increased migration or proliferation) to a wider spectrum of pro-angiogenic proteins (Fig. **2**) (TABLE 3).

Table 2. Impact of oncogenes or potential oncogenes on tumor angiogenesis. Assembled from references 22, 25, and 26.

Oncogene	Implicated pro-angiogenic activity
K-ras, H-ras	VEGF upregulation, TSP-1 downregulation
v-src	VEGF upregulation, TSP-1 downregulation
c-myb	TSP-2 downregulation
N-myc	angiogenic properties in neuroblastoma
c-myc	angiogenic properties in epidermis
HER-2	VEGF upregulation
EGFR	VEGF, bFGF, IL-8 upregulation
PyMT	TSP-1 downregulation
c-fos	VEGF expression
trkB	VEGF downregulation
HPV-16	Secretion of VEGF and IFN-a
v-p3k	VEGF production and angiogenesis
ODC	novel angiogenic factor
PTTG1	VEGF and bFGF upregulation
E2a-Pbx1	Induction of mouse angiogenin-3
bcl-2	VEGF upregulation

Table 3. Some examples of direct and indirect angiogenesis inhibitors. From reference 22.

Direct	Indirect
Angiostatin	Anti-VEGF antibody
Arresten	C225
Canstatin	Herceptin
Endostatin	Interferon-alpha
Thrombospondin	Iressa
TNP-470	NM-3
Tumstatin	PTK787
2-Methoxyestradiol	SU-5416
Vitaxin	SU-6668
	SU-11248

Selection of patients for a clinical trial of a "direct" angiogenesis inhibitor may in the future be based on analysis of the endothelial receptors in the patient's tumor. For example, the endothelial receptor for endostatin has recently been reported to be the integrin alpha5/beta1, while the endothelial receptor for tumstatin is alphav/beta3 [28].

Certain pro-angiogenic proteins also induce an increased level of circulating endothelial cells (or possibly progenitor endothelial cells derived from the bone marrow) which can be decreased toward normal by certain angiogenesis inhibitors [29]. If circulating endothelial cells can be shown to be a reliable surrogate marker for efficacy of an angiogenesis inhibitor, then clinical trials may be improved by a combination of (i) stratification of patients according to the pro-angiogenic protein(s) produced by their tumor; (ii) analysis of integrin receptors on endothelial cells in the vascular bed of the tumor, and (iii) quantification of circulating endothelial cells (by fluorescence activated cell sorting).

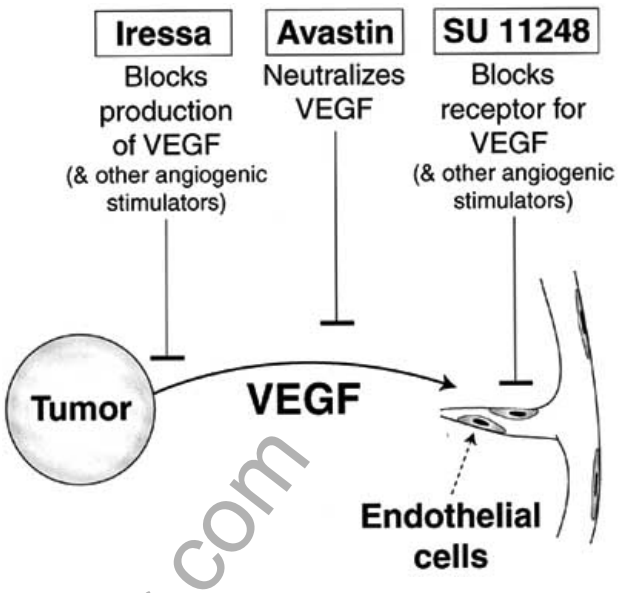

Figure 1. Examples of indirect angiogenesis inhibitors which can block vascular endothelial growth factor (VEGF). Iressa blocks VEGF production from the tumor, as well as blocking other pro-angiogenic proteins. Avastin neutralizes VEGF. SU11248 blocks the receptor for VEGF, as well as the receptors for other pro-angiogenic proteins.

In certain tumors, the angiogenic switch also involves down-regulation of endogenous angiogenesis inhibitors [30], in addition to increased expression of a pro-angiogenic protein. For example, *ras* transfection increases VEGF expression and decreases expression of thrombospondin [22]. In future clinical trials of angiogenesis inhibitors it may be helpful to monitor levels of endogenous angiogenesis inhibitors, but currently this is not as technically feasible as quantifying levels of pro-angiogenic proteins in tumors or in blood or urine [31].

ANGIOSTATIC STEROIDS: ANGIOGENESIS INHIBITORS WHICH ARE ENDOGENOUS

The demonstration that interferon alpha/beta inhibited endothelial cell motility *in vitro* [32] and the subsequent findings that interferon alpha inhibited angiogenesis *in vivo* in mice [33, 34], and in humans [35, 36], and inhibited tumor cell production of bFGF, [37] introduced the idea of the existence of natural

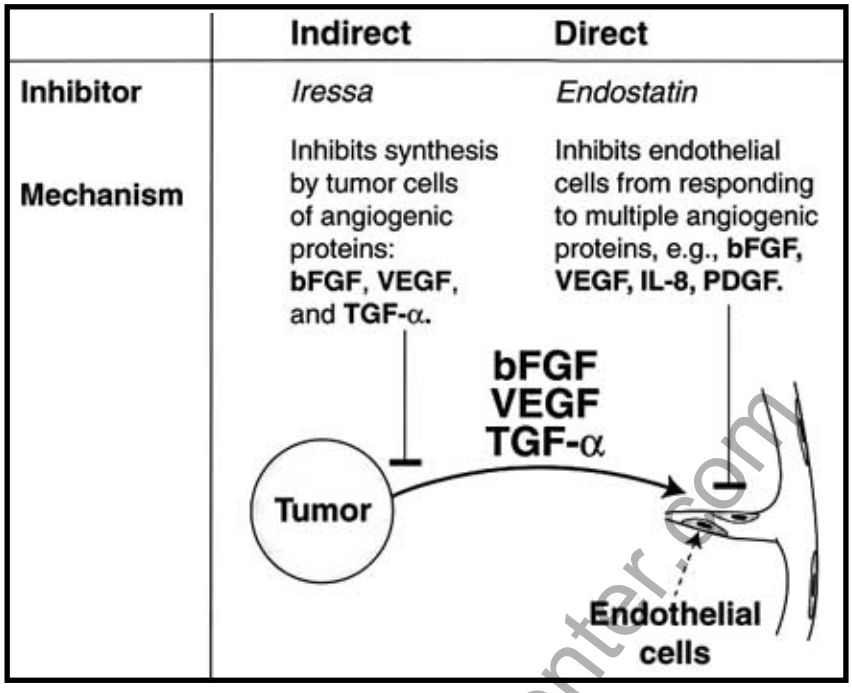

Figure 2. Direct and indirect angiogenesis inhibitors.
Direct angiogenesis inhibitors, such as endostatin, target the microvascular endothelial cells which are recruited to the tumor bed and prevent them from responding to various endothelial mitogens and motogens.
Indirect angiogenesis inhibitors, such as ZD1839 (Iressa), target oncogenes overexpressed by tumor cells, or the products of these oncogenes, or a receptor for these products. Therefore, Iressa targets the tyrosine kinase of the epidermal growth factor receptor and blocks its products bFGF, VEGF and TGF-α.

angiogenesis inhibitors in the body. The subsequent finding that certain corticosteroid metabolites, such as tetrahydrocortisol had antiangiogenic activity without mineralocorticoid or glucocorticoid activity, [38] further supported the concept of naturally occurring angiogenesis inhibitors, and paved the way for the discoveries of angiostatin, endostatin, and other angiogenesis inhibitors which are endogenous. A tetrahydrocortisol analogue is currently in a clinical trial for patients with macular degeneration. The existence of naturally occurring angiogenesis inhibitors which a tumor would have to overcome to induce angiogenesis also formed the basis for the subsequent concept of the 'angiogenic switch' [41, 24]. It is now recognized that at least two endogenous molecular barriers defend against pathological hotspots of angiogenesis: (i) angiogenesis inhibitors in the host such as tetrahydrocortisol, platelet factor 4, angiostatin, and endostatin; and (ii) angiogenesis inhibitors expressed by normal cells, but down-regulated during the switch to the angiogenesis phenotype in tumor cells, such as thrombospondin.

ANGIOGENIC PROTEINS ARE STORED IN EXTRACELLULAR MATRIX

After it was demonstrated that bFGF was stored in the cornea, bound to heparan sulfate in Descemet's membrane [39,40], it became clear that angiogenesis regulatory molecules are present in the body on a 'stand-by' basis. These pro-angiogenic proteins are maintained in a potentially active state and are releasable by specific enzymes or by heparin when angiogenesis is required in physiological processes such as reproduction or repair, and when angiogenesis is induced by pathological processes.

THE ANGIOGENIC SWITCH CONVERTS A NON-ANGIOGENIC MICROSCOPIC DORMANT TUMOR TO A VASCULARIZED GROWING TUMOR

The existence of natural endogenous angiogenesis inhibitors and of angiogenic proteins stored in the extracellular matrix, provided new insights which led to the concept of an angiogenic 'switch.' For a tumor to switch to the angiogenic phenotype, it must overcome two types of natural angiogenesis inhibitors (discussed above): (i) those inhibitors in the host's circulation or extracellular matrix; and/or (ii) those inhibitors in the tumor cell. Rastinejad *et al.* [30], demonstrated that tumor cells did not become angiogenic until they had significantly reduced their own production of thrombospondin. Bouck [41] proposed that the onset of angiogenesis was the result of a shift in the

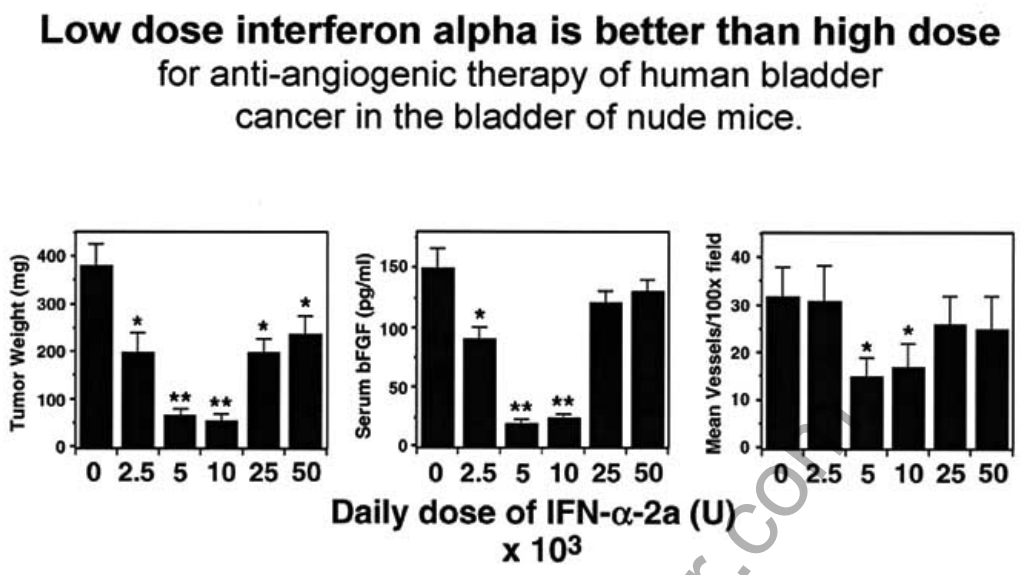

Figure 3. Systemic therapy of bladder tumors in mice with low-dose daily IFN-α-2a. Because of the U-shaped curve which is common to other cytokines, a low daily dose of interferon alpha is more effective than a high dose as an inhibitor of tumor growth, serum bFGF, or angiogenesis (mean vessel density).

balance of positive and negative regulators of angiogenesis. This shift takes place between the pro-angiogenic and anti-angiogenic proteins within the tumor cell itself, and between the tumor cell's angiogenic proteins and the hosts anti-angiogenic proteins [24]. A more detailed outline of the molecular and genetic mediators of the angiogenic switch is beyond the scope of this review, but is discussed in [22] and in [23].

The idea of the angiogenic switch [24] led to a new set of questions:

(i) What happens to a tumor that does not undergo the angiogenic switch? (ii) Can the angiogenic switch be prevented? The first question initiated a new line of research on the mechanism of the stability of the non-angiogenic phenotype in human tumors, i.e., the long time period during which *in situ*, microscopic non-angiogenic tumors remain dormant, (commonly called "no take" when human tumors are transplanted to immunodeficient mice) [12, 42]. Some of these tumors transplanted from human specimens, remain non-angiogenic indefinitely (i.e., more than a year in mice). Others spontaneously switch to the angiogenic phenotype in one to two months (Nava Almog and J. Folkman, unpublished data). Certain dormant human tumors can be rapidly switched to the angiogenic phenotype by transfection with the *ras* oncogene. This results in up-regulation of the tumor cell's production of VEGF and a decrease of its production of thrombospondin. It is now possible to isolate non-angiogenic clones of tumor cells from angiogenic human tumors removed surgically. A provocative finding is that virtually all human tumors examined so far contain significant numbers of non-angiogenic tumor cells. Therefore, the stage is set to experimentally test whether the angiogenic switch can be prevented in the non-angiogenic dormant *in situ* human tumor.

FIRST DEMONSTRATIONS IN MAN THAT HUMAN RECURRENT TUMORS CAN UNDERGO COMPLETE AND DURABLE REGRESSION BY TREATMENT WITH A SINGLE ANGIOGENESIS INHIBITOR

The first use of antiangiogenic therapy in a human was reported in 1989 by Carl White of Denver [35, 43]. (A very low dose of interferon alpha of 3 million units/meter^2/day subcutaneously was chosen during a telephone conversation between White and Folkman). A 12-year-old boy with fatal pulmonary hemangiomatosis had a complete remission and recovered completely after 7 months therapy. Therapy was continued for 7 years. He has been off therapy for 8 years, has graduated college and is working full time in finance. This led to the successful use of low dose daily interferon alpha therapy administered subcutaneously to infants with sight-threatening or life-threatening hemangiomas and hemangioendotheliomas of the heart, airway, and liver [44-48], and to the finding that these lesions over-expressed bFGF [49]. After these lesions underwent complete regression, they did not recur unless treatment time was less than 6 months, in which case treatment was resumed until complete regression.

This experience, coupled with the finding by Singh *et al.* [37] that interferon alpha suppressed expression and secretion of bFGF by human tumors, led us to the successful complete and durable

regression of recurrent high grade giant cell tumors and angioblastomas by treatment with low dose daily subcutaneous interferon alpha [31, 36, 50]. The fact that low dose interferon alpha was effective as an angiogenesis inhibitor in these patients was subsequently substantiated in pre-clinical models in Fidler's lab when they reported that the activity of interferon alpha on tumor growth, serum levels of bFGF and tumor microvessel density, followed a U-shaped curve [51] (see Fig. **3**).

The experience with low dose interferon alpha provided guidelines for subsequent clinical trials of antiangiogenic therapy in other types of cancer:

(i) Frequent dosing without off-therapy intervals is optimally effective.

(ii) Low dose is better than high dose.

(iii) Long-term therapy is necessary, because antiangiogenic therapy is slower than conventional chemotherapy.

(iv) Long-term therapy is feasible because side-effects of antiangiogenic therapy are generally less than with conventional chemotherapy.

(v) A surrogate marker (bFGF in this case), for efficacy of antiangiogenic therapy is valuable for dose adjustment.

(vi) Complete and durable regression was common because the patients were selected to have tumors which produced a single angiogenic protein, bFGF. The expression of this angiogenic protein was inhibited by low dose interferon alpha, frequently administered.

ANEUPLOIDY MAY BE CAUSED BY HORIZONTAL TRANSMISSION OF GENETIC INFORMATION WHEN A TUMOR CELL PHAGOCYTOSES AN APOPTOTIC BODY

One fundamental difference between normal cells and neoplastic cells is that normal cells are genetically stable and maintain a normal karyotype. In contrast, tumor cells are genetically unstable. In the cells of most common human tumors, there is an abnormal karyotype from one cell to another. This genetic instability contributes to the high risk of acquired drug resistance when any therapy is used which attacks the tumor cell *per se*. Antiangiogenic therapy, however, is based on treating a stable genetic target, the activated microvascular endothelial cell in the tumor bed [52].

The chaotic karyotype of cancers cells is characterized by aneuploidy. Recently, Lars Holmgren discovered a novel mechanism of aneuploidy while working as a post-doctoral fellow in the Folkman lab [53]. He continued to study this phenomenon and subsequently demonstrated that while most neoplastic cells which phagocytose an apoptotic body of a neighboring cell, can incorporate DNA and its genetic information by horizontal transfer, apparently only those tumor cells which are p53 -/- can vertically transmit this new genetic material to daughter cells [54]. Cells with functioning p53 appear to be killed when they try to proliferate while carrying abnormal DNA.

PRIMARY TUMORS CAN SUPPRESS GROWTH OF SECONDARY METASTASES BY AN ANGIOGENIC MECHANISM

It is well known among surgeons that removal of certain primary tumors may lead to rapid growth of secondary metastases, reviewed in [55]. A novel mechanism to explain this has been reported [55, 56, 11]. Primary tumors express and secrete high levels of pro-angiogenic proteins, e.g., VEGF. But, these tumors also generate angiogenesis inhibitors by enzymatic cleavage of cryptic fragments from large proteins, (i.e., angiostatin from plasminogen and endostatin from collagen XVIII). Because VEGF has a short half-life in the circulation (minutes) and the angiogenesis inhibitor proteins have a longer half-life in the circulation (hours), the inhibitor accumulates in the plasma in excess of the stimulator and prevents the induction of angiogenesis by micrometases already in place. In the primary tumor however, the angiogenic stimulator (e.g., VEGF) would be in excess of the inhibitor, thus allowing continued growth of the primary tumor while the secondary metastases are suppressed. Surgical removal of the primary tumor causes a decrease in circulating angiogenesis inhibitor with a resultant induction of angiogenesis in pre-existing micrometastases. The same effect can be demonstrated when a primary tumor is regressed by ionizing irradiation [57]. Additional evidence for this concept is provided by experiments in which the inhibitor (angiostatin) is overexpressed by gene transfer in all tumor cells [58].

The elucidation of this phenomenon led to the discovery of the first cryptic angiogenesis inhibitors which are endogenous. It also provided an explanation of the experimental phenomenon of "concomitant tumor resistance," in which tumor cells are inoculated on both flanks of a mouse, but only one tumor grows. When that tumor is surgically removed, the tumor on the opposite flank grows. It also provides a biological explanation for 4 common patterns of presentation of metastases, either by angiogenic regulation of metastatic dormancy or by escape from it [59].

CRYPTIC FRAGMENTS OF MATRIX PROTEINS ARE SPECIFIC INHIBITORS OF ANGIOGENESIS

The discovery of endostatin [56], revealed that a cryptic fragment of collagen XVIII inhibited angiogenesis, whereas the parent protein did not. This suggests a role for matrix proteins in the regulation of angiogenesis [60]. The discovery of

tumstatin in collagen IV by Kalluri's lab [61, 62], further implicated basement membrane proteins as regulators of angiogenesis.

When these proteins are considered together with angiostatin, and antiangiogenic anti-thrombin III [63] a new paradigm is revealed that some proteins (e.g., plasminogen and basement membrane) harbor "unique properties that are cryptic and become exposed only upon proteolytic degradation" [23].

Mice from which tumstatin has been deleted demonstrate the effect of the loss of a cryptic angiogenesis inhibitor. Tumstatin is contained in the alpha 3 chain of collagen IV. When the gene for this chain is knocked out, tumstatin blood levels in mice which are normally in the range of 336 +/- 28 ng/ml decrease to 0 ng/ml and tumors grow 300% to 400% faster, reaching 7 cm^3 in 26 days [64]. However, if tumstatin is replaced at physiological levels (i.e. to achieve normal blood levels), tumors return to the same slower growth rate as in the wild type mice. This fulfills the classic paradigm of a tumor suppressor protein (like p53), except that tumstatin is purely antiangiogenic and has no other known functions). In Kalluri's studies [64] the angiogenesis inhibitor (the ligand) has been knocked out, its receptor has been knocked out, and the enzyme which releases the ligand from its matrix has been knocked out. All models predictability show the same effect of increased angiogenesis, followed by increased tumor growth. Wound healing and pregnancy are not affected. These experiments provide genetic evidence that a normal physiological function of an endogenous angiogenesis inhibitor may be to defend against pathological angiogenesis.

LEUKEMIA IS ANGIOGENIC

The demonstration that human leukemia and other hematological malignancies are angiogenic (in vivo) [65-73], expanded the concept that tumors are angiogenesis-dependent, to the possibility that leukemia and other hematological malignancies may also be angiogenesis-dependent. Further evidence for this hypothesis has recently been published [65] (for review see Folkman and Kalluri Cancer Medicine page 734). A recent study reported that retroviral gene transfer of a vector encoding the direct angiogenesis inhibitors angiostatin and endostatin inhibited bone-marrow angiogenesis and tumor growth in a mouse model of leukemia [74]. Mice inoculated with B-cell, T-cell or myelogenous leukemias and treated with recombinant endostatin have also been observed to live significantly longer and experience fewer toxic side effects than with conventional chemotherapy (Timothy Browder et al, unpublished studies). Taken together, these data suggest that leukemias may be angiogenesis-dependent and may be susceptible to antiangiogenic therapy.

ENDOTHELIAL CELLS APPEAR TO CONTROL SIZE OF NORMAL TISSUE MASS

If microvascular endothelial cells control the growth of virtually all malignancies, we can ask if endothelial cells also control growth of normal tissue mass. Three pieces of experimental evidence indicate that they do. Testosterone induces the up-regulation of VEGF in prostate which leads to angiogenesis in the gland [75]. Proliferating endothelial cells release a variety of mitogens and survival factors which may coordinate prostate hyperplasia with the growth of its endothelial cell population. Furthermore, a natural endogenous angiogenesis inhibitor, pigment epithelium – derived factor (PEDF), regulates the vasculature and mass of the prostate and pancreas [76]. In leptin knockout mice gain of body fat increases continuously and is accompanied by parallel growth of capillary blood vessels. When these mice are treated with an angiogenesis inhibitor (e.g. endostatin or TNP-470), endothelial cells in the adipose tissue undergo apoptosis. The adipose tissue involutes and mice lose weight steadily (but stop losing weight at a physiologically appropriate weight for age [77]). Liver regeneration after hepatectomy in mice has also been reported to be associated with angiogenesis or is angiogenesis-dependent [78-80]. Therefore, it is possible that all tissue mass, whether it is neoplastic or normal, may be regulated by microvascular endothelial cells.

OPTIMUM ANTIANGIOGENIC THERAPY IS ACHIEVED BY CONTINUOUS LEVELS OF ANGIOGENESIS INHIBITOR

A major difference between conventional chemotherapy and antiangiogenic therapy is that chemotherapy has traditionally been administered at maximum tolerated doses with off-therapy intervals of days to weeks, designed to rescue bone marrow and to allow restoration of gastrointestinal epithelium. However, optimum antiangiogenic therapy provides a continuous blood level of the angiogenesis inhibitor. The rationale for this is that tumor-derived pro-angiogenic molecules which continually bathe microvascular endothelial cells, will be opposed by angiogenesis inhibitor molecules. The most compelling of many experiments which support this concept is that continuous administration of endostatin by a micro-osmotic pump in the peritoneal cavity of mice bearing subcutaneous human pancreatic cancer, was 10-fold more effective at inhibiting tumor growth than the same dose given as a bolus injection once per day [81]. Continuous therapy led to tumor regression, bolus once/day therapy did not. Patients receiving endostatin subcutaneously twice daily by a subcutaneous, sustained-release formulation, attain steady state blood levels which are very similar to those attained by continuous intravenous therapy.

CYTOTOXIC CHEMOTHERAPY IS ANGIOGENESIS-DEPENDENT, IN PART

If antiangiogenic therapy is best administered on a schedule that permits continuous exposure of the activated microvascular endothelium in the tumor bed to angiogenesis inhibitor(s), would administration of conventional chemotherapy on a similar schedule improve efficacy of chemotherapy or convert a drug resistant tumor to a drug responsive tumor? Timothy Browder in the Folkman lab answered this question by mouse experiments in which tumors which had failed to respond to "conventional scheduling" of cyclophosphamide (i.e., every other day for 3 days, followed by 21 days off therapy and then the cycle was repeated), regressed when mice were treated on an antiangiogenic schedule of cyclophosphamide administered every 6 days at a lower total dose [82]. Endothelial cell apoptosis in the tumor bed was followed within 4-5 days by apoptosis of tumor cells surrounding each capillary with apoptotic endothelial cells. This report was confirmed and extended by Kerbel's lab [83], using etoposide instead of cyclophosphamide. In a subsequent editorial by Douglas Hanahan [84], this approach was termed "metronomic" chemotherapy. The mechanism of endothelial apoptosis in low dose (metronomic) (anti-angiogenic) chemotherapy remains to be elucidated. Nevertheless, it will be interesting to see if this change in dose and schedule of conventional chemotherapy will by-pass drug resistance because the endothelial cell is the direct target of therapy instead of the cancer cell.

CANCER MAY BE CONVERTED TO A CHRONIC MANAGEABLE DISEASE

As angiogenesis inhibitors become more widely used in anti-cancer therapy, It will be important to determine: (i) Can the harsh side-effects of conventional chemotherapy be reduced? (ii) Can the risk of drug resistance be reduced? (iii) Can cancer eventually be converted to a chronic manageable disease, like heart disease or diabetes? [85]

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