British Journal of Surgery 1998, 85, 1044–1055

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Review

Significance of angiogenesis in cancer therapy

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Background For most solid tumours, surgery remains the most effective primary treatment. Despite apparently curative resection, significant numbers of patients develop secondary disease due to growth of undetected micrometastases. The ability of a tumour to metastasize is related to the degree of angiogenesis it induces. In addition, micrometastases rely on new vessel formation to provide the nutrients necessary for growth. A better understanding of how tumours acquire their blood supply may lead to more effective adjuvant therapies and improve survival following surgery.

Methods A systematic review of the literature on angiogenesis between 1971 and 1997 was performed using the Medline database to ascertain current thinking on angiogenesis and its relevance in oncological surgery.

Results Angiogenesis is a physiological process subject to autocrine and paracrine regulation which has the potential to become abnormal and play a part in a number of pathological states, including cancer. Increased angiogenic stimuli in the perioperative period, associated with concomitant reduction in tumour-derived antiangiogenic factors following resection of a primary tumour, result in a permissive environment which allows micrometastases to grow.

Conclusion Recognition of the role of angiogenesis in metastatic tumour growth represents a significant development in our understanding of tumour biology. The development of antiangiogenic agents offers new promise in the treatment of malignancy. Such agents may prevent or control the development and growth of primary and metastatic tumours.

Angiogenesis is a process through which new blood vessels develop from pre-existing vessels such as capillaries and postcapillary venules[1,2]. It is tightly regulated by a large number of proangiogenic and antiangiogenic factors. In physiological circumstances, angiogenesis is fully controlled by an equilibrium between these factors such that little blood vessel growth occurs in the healthy adult. Accelerated angiogenesis is a normal physiological response to wound healing, inflammation, menstruation and embryonic development, but a pathological feature of conditions such as diabetic retinopathy, rheumatoid arthritis and solid tumours.

The pivotal role of angiogenesis in primary tumour growth and metastasis has been recognized for many years, although the mechanisms which control it are incompletely understood[3]. Growth of a tumour beyond 2–3 mm^3 requires development of a microvessel network to facilitate delivery of nutrients and oxygen, and removal of catabolites. Density of microvasculature has been used as an indicator of biological aggressiveness and metastatic potential in many primary tumours[4], as neovascularization facilitates metastasis[1,5] by providing access to the circulation[6,7]. The degree of angiogenesis in primary breast[8], prostate[4] and colorectal[9] carcinoma correlates with lymph node metastasis. The predictive value of microvessel density in the primary tumour remains controversial. Microvessel density has been shown to be an independent prognostic indicator in non-small cell lung cancer[10], certain instances of node-negative breast cancer[11,12] and several other carcinomas[13]. However, conflicting reports exist, particularly in relation to its prognostic value in breast cancer[14,15]. These conflicting findings may be attributable, in part, to the heterogeneity of microvessel density within individual tumours, as well as to interlaboratory and interobserver variability in microvessel counting[12].

Our understanding of mechanisms regulating angiogenesis in metastatic disease is increasing[16]. Acquisition of a blood supply by micrometastases at the site of implantation is crucial to tumour growth. Neovascularization is promoted by a number of proangiogenic factors of tumour and stromal origin. The clinical impression that resection of a primary tumour heralds a phase of increased metastatic growth is of particular interest, but until recently no explanation of this phenomenon existed. Recent studies have shown that the intact primary tumour can regulate growth of associated metastases, either directly or indirectly, through the production of certain antiangiogenic factors, notably angiostatin[17]. Surgical excision of a primary tumour removes the source of the inhibitory angiostatin and other factors, allowing angiogenesis and subsequent growth of previously dormant micrometastases[18]. Tumour recurrence many years after apparently successful treatment of a primary lesion is partly the result of increased angiogenesis[19,20]. Long-term suppression of angiogenesis may become a therapeutic option for induction of long-term remission by maintaining micrometastases in a state of dormancy, a dynamic equilibrium during which there is no net tumour growth[20]. The proliferating capillary endothelial cell offers a unique target for antiangiogenic therapy[21] as antiangiogenic strategies may reduce both the recurrence rate and the metastatic potential of solid tumours[22].

Paper accepted 9 March 1998

Stages of neovascularization

Newly formed capillaries are composed of two cell types, endothelial cells and pericytes. These two cells have the capacity to produce entire capillary networks. Following the transduction of signals which promote differentiation *in vivo*, angiogenesis progresses in four stages: activation of endothelial cells, proliferation, migration and lumen formation[23].

Cytokine release is provoked by factors endogenous and exogenous to tumour cells, including local environmental factors such as hypoxia. Quiescent endothelial cells are activated by the release of cytokines from host and tumour cells (stage 1). Committed cells proliferate (stage 2), then migrate along a fibrin skeleton towards the source of the angiogenic stimulus to form cords of aligned cells (stage 3). Finally, the vascular sprout forms a lumen and the cells exit the cell cycle to a resting phase. Development of a patent lumen (stage 4) occurs through coalescence of intracellular vacuoles and is facilitated by cell-to-cell adhesive contact[1].

Degradation of the extracellular matrix is an essential component of new vessel invasion. This is facilitated by alteration of the proteolytic balance. Proteolytic degradation of the extracellular matrix and fibrinolysis are two functions of pericytes[24], although pericytes are also thought to contribute by production of growth factors and growth inhibitors. Cell adhesion receptors promote vascular cell migration through interaction with adhesion proteins of the extracellular matrix, such as collagen and fibronectin. The extracellular matrix also serves as a reservoir for growth factors, particularly acidic fibroblast growth factor and basic fibroblast growth factor (bFGF)[25–27].

The capillary sprouts in tumours are 'leaky' as proliferating capillaries have incomplete basement membranes. In addition, vascular endothelial growth factor (VEGF; previously known as vascular permeability factor) increases permeability through the development of a series of interconnected cytoplasmic vesicles and vacuoles (known as vesical-vacuolar organelles) that maintain contact with both the luminal and abluminal surface[28,29]. In normal tissues this may play a regulatory role in controlling baseline microvascular permeability[30], and in tumour microvasculature this feature has been linked to malignant exudates and ascites[31,32].

Regulation of angiogenesis

The degree of angiogenesis in a tumour is the result of complex interactions between tumour cells, capillary endothelial cells, pericytes and recruited immune cells with products of these cells acting in both an autocrine and paracrine fashion[33]. It is normally subject to tight physiological control through a balance of proangiogenic and antiangiogenic factors. These factors allow a phase of rapid proliferation to facilitate wound healing but maintain quiescence in the mature microvasculature[5]. Increased production of positive angiogenic factors is 'necessary but not sufficient'[34] for induction of the angiogenic phenotype; negative regulators must also be decreased[18,35,36]. Imbalance between angiogenic promoters and inhibitors produces the intense angiogenesis which is characteristic of many pathological processes, including diabetic retinopathy[37–39], rheumatoid arthritis[40], endometriosis[41] and malignant tumours[29,42].

Factors influencing angiogenesis are derived both from tumour cells and infiltrating cells, such as macrophages and fibroblasts[7]. Through their secretory products, activated macrophages can influence each phase of the angiogenic process[43–45]. The process of macrophage activation is mediated in part by hypoxia[46]. The large number of macrophages present in a variety of angiogenesis-associated pathological states is indicative of their influence. It is known, for example, that numerous macrophages are present in the first phase of tumour growth. Macrophage density is directly proportional to rate of tumour growth in breast carcinoma[47]. Furthermore, tumour-associated macrophages cause marked augmentation of tumour neovascularization[40] and correlate directly with prognosis in this disease[48]. Other immune cells are also significant, as neutrophilia is associated with a poorer prognosis in breast cancer[49].

Proangiogenic factors (*Table 1*)

Vascular endothelial growth factor

VEGF is the most potent directly acting angiogenic protein known[50]. It is a diffusible endothelial cell-specific

Table 1 Proangiogenic substance

Substance	Reference	
Vascular endothelial growth factor	Marme[50]	1996
Basic fibroblast growth factor (FGF-2)	Rifkin and Moscatelli[74]	1989
Acidic fibroblast growth factor (FGF-1)	Jouanneau *et al.*[177]	1995
Platelet-derived endothelial cell growth factor	Takahashi *et al.*[7]	1996
Platelet activating factor	Camussi *et al.*[178]	1995
Transforming growth factor β1	Pepper *et al.*[102]	1993
Transforming growth factor α	Gleave *et al.*[179]	1993
Insulin-like growth factor	Nicosia *et al.*[180]	1994
Epidermal growth factor	Goldman *et al.*[181]	1993
Interleukin 1	Fan *et al.*[182]	1993
Interleukin 4	Wojta *et al.*[183]	1993
Interleukin 6	Sunderkotter *et al.*[43]	1994
Interleukin 8	Smith *et al.*[184]	1994
Interleukin 15	Angiolillo *et al.*[185]	1997
Angiogenin	Moenner *et al.*[186]	1994
Vascular integrins $\alpha v_1 \beta_3$ and $\alpha v_1 \beta_5$	Varner and Cheresh[1]	1996
Fibronectin	Bitterman *et al.*[26]	1983
Fibrin	Tsopanoglau *et al.*[187]	1993
Tumour necrosis factor α (*in vivo*)	Frater-Schroder *et al.*[111]	1987
Endotoxin (lipopolysaccharide)	Mattsby-Baltzer *et al.*[112]	1994
Matrix metalloproteinases	Cornelius *et al.*[188]	1995
Scatter factor (hepatocyte growth factor)	Rosen *et al.*[189]	1993
Hyaluron fragments	Cockerill *et al.*[23]	1995
Granulocyte–macrophage colony stimulating factor	Bikfalvi and Han[190]	1994
Platelet activating factor	Bussolino *et al.*[191]	1995
Proliferin	Jackson *et al.*[192]	1994
Substance P	Fan *et al.*[182]	1993
Lactate	Magee *et al.*[193]	1994
Prostaglandin E_1, E_2	Ben-Av *et al.*[194]	1995
Urokinase plasminogen activator	Hildenbrand *et al.*[195]	1995
Tenascin	Canfield and Schor[196]	1995
Nitric oxide	Ziche *et al.*[197]	1994
Erucamide	Mitchell *et al.*[198]	1996

mitogen and angiogenic factor, which also increases vascular permeability[51,52]. It binds to two endothelial cell tyrosine kinase receptors, the fms-like tyrosine kinase (flt) and the kinase domain receptor (KDR)[53,54].

Tumour-derived VEGF plays an important role in the paracrine stimulation of angiogenesis but it also appears to have an autocrine stimulatory effect on tumour cells[55], particularly in response to hypoxia[56]. Hypoxia stimulates angiogenesis in a number of sites, including endothelial cells, retinal pericytes[57], myocardium[58] and solid tumours[59]. VEGF activity is potentiated by oxygen deprivation[60–62], mediated in part by adenosine[57,63] through upregulation of the VEGF endothelial cell receptor KDR[64]. Wild-type *p53*, a tumour suppressor gene, inhibits proliferation of both normal and transformed cells[65,66], and also regulates VEGF production. Mutations in this gene abolish such control. There appear to be two regulatory VEGF pathways: an oncogenic one (v-*src*) that enhances VEGF production and a tumour suppressor (*p53*) signal that suppresses VEGF[50]. Mutations of the *ras* oncogene cause VEGF upregulation in a colon cancer model with resulting increased angiogenesis[67]. H-*ras* oncogene mutation also activates angiogenesis through upregulation of VEGF and matrix metalloproteinase (MMP) bioactivity, while downregulating activity of tissue inhibitors of MMP[68].

Tumour cell lines which express minimal constitutive and hypoxia-inducible VEGF (e.g. human melanoma SK-MEL-2 cells) produce small, poorly vascularized tumours. Overexpression of VEGF results in dramatically increased angiogenesis and subsequent tumour expansion[69]. Interruption of the VEGF receptor paracrine pathway in glioma tumours inhibits angiogenesis, leading to reduced numbers of new vessels, a higher degree of necrosis and a reduction in size of glioma tumours[70]. Expression of VEGF and its receptor KDR is higher in metastatic than in non-metastatic colonic neoplasms, and correlates directly with the extent of neovascularization and degree of proliferation[9]. There is a direct correlation between serum VEGF concentration and breast cancer stage[71].

Soluble recombinant flt receptor binds to free VEGF and inhibits its mitogenic affinity for endothelial cells[72]. Anti-VEGF antibodies have been used as a new therapeutic modality for the prevention of retinal ischaemia-induced neovascularization of the iris, confirming that the *in vitro* effects of VEGF inhibition are reproducible *in vivo*[73].

Basic fibroblast growth factor

This is a 16-kDa protein which is one of a family of fibroblast growth factors[74]. Capillary endothelial cells themselves produce and release bFGF which acts in an autocrine fashion independently, and synergistically with VEGF[75], as an endothelial cell mitogen[76]. It binds intensely to the extracellular matrix, but is normally confined to its cell of origin as it lacks the signal peptide thought to be necessary for secretion[50]. During the malignant evolution of fibrosarcoma, neovascularization is associated with a switch to the ability to export bFGF from cells[77]. It also stimulates endothelial and vascular smooth muscle cell motility[78,79] especially after arterial trauma[80]. Heparin released by recruited mast cells amplifies its effect[5]. Its systemic administration results in an increased rate of tumour growth and increased vascularity[81]. Binding by bFGF is inhibited by suramin, an antiparasitic agent[82]. It has been shown that blockade of bFGF receptors results in suppression of solid tumour growth[83].

Acidic fibroblast growth factor

This is a heparin binding site-specific endothelial cell mitogen[84,85] which promotes angiogenesis and wound healing[86,87]. It is expressed constitutively in normal as well as in tumour cells, but appears to be inhibited by an angiogenesis suppressor gene which is downregulated during tumorigenesis[34]. Like bFGF, it is found in many normal cells but exported only in tumour cells.

Platelet-derived endothelial cell growth factor

This proangiogenic cytokine is found in platelets[88] and released during the blood clotting cascade, as well as being produced by vascular smooth muscle cells[89]. It is an endothelial cell mitogen but is not a classical growth factor, because direct contact with a cell-surface receptor is not required for its mitogenic capability[90]. It promotes endothelial cell migration[91] and may facilitate endothelial differentiation[92]. Platelet-derived endothelial cell growth factor (PD-ECGF) expression is known to be modulated by the tumour microenvironment[93]. It is homologous to thymidine phosphorylase[94], an enzyme which catalyses the reversible breakdown of thymidine to thymine and deoxyribose-1-phosphate. This enzymatic activity may contribute to its angiogenic activity, as products of deoxyribose-1-phosphate are known to be angiogenic, although the mechanism through which PD-ECGF exerts is angiogenic effect is not yet fully understood[95,96].

Transforming growth factor β

Transforming growth factor (TGF) β, which is produced by macrophages and activated platelets, exists in three human isoforms, β1, β2 and β3. All act as chemotactic agents for macrophages and are indirectly angiogenic *in vivo*[97–101], although TGF β1 appears most potent in this regard[100]. TGF β1 facilitates wound healing through accelerated collagen deposition and maturation[101]. It induces matrix production and alters integrin production to facilitate neovascularization. It acts in a dose-dependent manner, with low concentrations exhibiting stimulatory and high concentrations inhibitory effects[102]. Its absence results in poor vascular integrity and reduced remodelling[103]. TGF β3 appears to have a greater role in matrix remodelling, with a resultant decrease in scar formation[100].

Epithelial growth factor

Epidermal growth factor (EGF) is a mitogenic growth factor. EGF receptors belong to a group of proto-oncogenes, including c-*erbB2*, which are overexpressed in a number of human tumours[104]. EGF is secreted by tumour-associated macrophages in patients with carcinoma of the breast[105].

Integrins

Blood vessels involved in angiogenesis express adhesion proteins, known as integrins, including von Willebrand factor, fibronectin, fibrin and vascular integrin $\alpha v_1 \beta_3$ and $\alpha v_1 \beta_5$. They facilitate cell–cell and cell–matrix interactions[106], thereby playing a crucial role in endothelial

cell chemotaxis. Adhesion proteins may be selectively inhibited by monoclonal antibodies with the resulting decrease in angiogenesis inducing apoptosis of endothelial cells[34]. Vascular integrin $\alpha v_1 \beta_3$ is selectively expressed on growing vessels, where it suppresses p53 activity while increasing the *bcl2*:*bax* ratio with a net proangiogenic and antiapoptotic effect[107]. Its inhibition by monoclonal antibodies in a melanoma model results in reduced angiogenesis, suggesting that the new tumour vasculature grows in a self-perpetuating manner[108].

Tumour necrosis factor α

Tumour necrosis factor (TNF) α is a macrophage-derived angiogenic factor which is considered to be the principal mediator of tumour cell cytotoxicity and cytostasis[109]. It has a broad spectrum of biological activities including both stimulatory and inhibitory effects on target cells which are dose related[40,110]. It induces granulocyte–macrophage colony stimulating factor production, has procoagulant activity and increases the adherence of human endothelial cells. Its proangiogenic activity was initially postulated to be induced through an inflammatory mechanism secondary to its cytotoxic effects[111]. It is now believed that TNF-α is a potent mediator of angiogenesis at low concentrations, independent of inflammation[110].

Endotoxin

Endotoxin (lipopolysaccharide; LPS) is a ubiquitous molecule derived from the cell wall of most Gram-negative and some Gram-positive bacteria as well as existing in a free form in the environment. It is a proangiogenic molecule because of its ability to elicit production of a wide spectrum of host-effector molecules, such as VEGF, bFGF, TGF-β, TNF, interleukin (IL) 1 and IL-6, by macrophages and other cell populations[112–114]. Recent work from the authors' laboratory shows that LPS introduced at the time of open surgery in a tumour-bearing murine model increases serum VEGF concentration[115].

Antiangiogenic factors (*Table 2*)

Thrombospondin

Thrombospondin is a glycoprotein secreted by many cells, including endothelial and epithelial cells, fibroblasts, smooth muscle cells, monocytes and macrophages[116]. It modulates endothelial cell adhesiveness, motility and proteolytic activity by sequestering angiogenesis inducers[117]. While it is a potent inhibitor of angiogenesis, it is downregulated during tumorigenesis[5,118,119]. In fibroblasts, wild-type p53 inhibits angiogenesis through upregulation of thrombospondin[33], an effect which is abolished by p53 mutations. Paradoxically, thrombospondin enhances the angiogenic effect of LPS and bFGF stimuli in inflammatory states[120]. It is speculated that thrombospondin 1 downregulates VEGF expression through an as yet unknown mechanism[48].

Angiostatin

Angiostatin is a 38-kDa internal fragment of plasminogen[121] which inhibits endothelial cell proliferation, angiogenesis and tumour growth. It blocks the catalytic site of the enzyme that cleaves plasminogen, thereby preventing the matrix remodelling required for angiogenesis[117]. It selectively prevents endothelial cells responding to angiogenic stimuli resulting in dormancy of micrometastases[122]. Systemic administration has been shown to induce regression of primary tumours of breast, colon and prostate in a murine model, providing the first example of dormancy therapy in which tumours undergo regression through blockade of angiogenesis[17].

Table 2 Antiangiogenic substances

Substance	Reference	Year
Thrombospondin	Canfield and Schor[196]	1995
Angiostatin	O'Reilly *et al.*[121]	1994
Endostatin	O'Reilly *et al.*[123]	1997
Suramin	Takano *et al.*[170]	1994
Interferon α and β	Stout *et al.*[168]	1993
Interferon γ	Kobayashi *et al.*[199]	1995
Interferon μ inducible protein 10	Angiolillo *et al.*[200]	1996
Interleukin 12	Voest *et al.*[169]	1995
Tamoxifen	Donovan *et al.*[32]	1997
Thalidomide	D'Amato *et al.*[158]	1994
Linomide	Vukanovic and Isaacs[201]	1995
Fumagillin analogues (AGM-1470)	Klauber *et al.*[160]	1997
Captopril	Volpert *et al.*[202]	1996
Platelet factor 4	Strieter *et al.*[203]	1995
Antisense VEGF RNA, VEGF blocking antibody and VEGF receptor kinase inhibitors	Millauer *et al.*[204]	1996
Transforming growth factor β1	Pepper *et al.*[102]	1993
Proliferin-related protein	Jackson *et al.*[192]	1994
2-methoxyoestradiol	Fotsis *et al.*[205]	1994
Tissue inhibitor of metalloproteinase	Tagikawa *et al.*[206]	1990
Retinoids	Pienta *et al.*[207]	1993
Tumour necrosis factor α (*in vitro*)	Frater-Schroder *et al.*[111]	1987
Group B *Streptococcus* toxin CM 101	Thurman *et al.*[208]	1996
Pentosan polysulphate	Nguyen *et al.*[209]	1993
Lavendustin A	Hu and Fan[210]	1995
Dexamethasone	Hori *et al.*[211]	1996
Medroxyprogesterone acetate	Yamamoto *et al.*[212]	1994
Korean misletoe extract (*Viscum album coloratum*)	Yoon *et al.*[213]	1995
Indomethacin	Tarnawski *et al.*[214]	1989
Diclofenac	Lala *et al.*[215]	1997

VEGF, vascular endothelial growth factor

Endostatin

Endostatin is a protein fragment of collagen XVIII and was first isolated from a murine haemangioendothelioma cell line[123]. It acts in a similar way to angiostatin, blocking collagenase and impeding matrix remodelling. It specifically inhibits endothelial cell proliferation. Recombinant endostatin inhibits angiogenesis, growth of metastases and growth of primary tumours[123]. Endostatin-treated tumours have a proliferation rate which is the same as that of untreated tumours, but an apoptotic rate which is sevenfold greater, providing a further example of dormancy therapy.

Tumour angiogenesis

Malignant transformation is a cumulative process requiring loss of control of the cell cycle and a shift in the

balance of proangiogenic and antiangiogenic factors. Induction of angiogenesis is a local event which is specific to activated hyperplastic cells and which procedes overt tumour formation[124]. For example, angiogenesis is a marker of premalignant transformation in benign breast disease[125,126]. Vascular endothelial cells in malignant breast tumours express tissue factor, a potent procoagulant, whereas vascular endothelial cells in benign tumours do not[127]. Cell proliferation and malignant transformation represent a switch to an angiogenic phenotype, but only in the latter is control of the cell cycle lost. Furthermore, only those hyperplastic cells which acquire angiogenic capacity undergo malignant transformation[124,126]. One model involves a tumour suppressor gene encoding the angiogenesis inhibitory factor thrombospondin, which is downregulated when cells undergo malignant transformation, allowing the angiogenic phase to commence[118]. This downregulation is under the control of the *p53* tumour suppressor gene[33]. Loss of suppressor gene sequences[128] leads to failure of inhibitory pathways with resultant tumour growth[110].

Numerous parallels between wound healing and tumour growth exist, suggesting that tumours act as 'obligate parasites' which have developed the capacity to pre-empt and subvert the wound healing response of the host as a means to acquire the stroma they need to grow and expand[129]. Normal cells involved in wound healing, such as endothelial cells and fibroblasts, develop features similar to those of malignant cells[130]. These features include increased proteolysis[131], increased motility[132] and proliferation. It is postulated that the invasive, proliferative behaviour of transformed cells may result from a functional failure of signals that usually regulate the activated behaviour of normal cells in a wound[129].

Angiogenesis and tumour metastasis

Tumour cells must detach from a primary tumour to initiate the 'metastatic cascade'[19,133]. Angiogenesis is a necessary precursor of metastasis as new proliferating capillaries have incomplete basement membranes and are 'leaky', facilitating penetrance by tumour cells[134,135]. These tumour cells must evade immune surveillance in the circulation and also at the site of secondary development, where they cross endothelium, degrade basement membrane, implant, proliferate and establish their own capillary network[19]. Angiogenesis is necessary at both the beginning and the end of the metastatic cascade. There is resultant metastatic inefficiency as only a small number of tumour cells which leave the primary tumour successfully implant at distant sites[136,137].

Effect of surgery on angiogenesis

Clinical experience and experimental evidence support the concept that the healing wound is an immunologically privileged site for metastases[25,106,138,139]. The cytokine environment that ensues in the first 24 h following surgical trauma influences the healing process for the subsequent 3 weeks[101]. Tumour growth is enhanced in healing wounds, but not in surrounding normal tissues. It has been shown that when tumour cells reach a healing colonic anastomosis or laparotomy wound within 2 h of its formation, the probability of a tumour cell leading to a metastatic deposit is increased 1000-fold compared with normal tissue[140]. Evidence showing an increased hazard rate for relapse or death in the 3 years following surgery for breast cancer, and a second peak a number of years later, supports the theory that micrometastases with a variable rate of growth are present at the time of diagnosis[141]. Surgery and subsequent healing may contribute to the genesis of recurrent disease through promotion of a proangiogenic climate which alters the dynamic equilibrium that would otherwise maintain micrometastases in a state of dormancy[20,142] (*Fig. 1*).

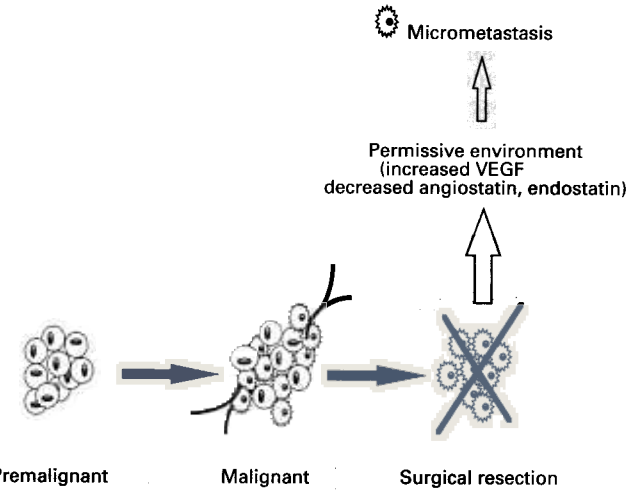

Fig. 1 Effect of surgery on micrometastases. VEGF, vascular endothelial growth factor; LPS, lipopolysaccharide

VEGF may be one of the primary links between these observations, as it is necessary to allow wound healing but may also foster growth of micrometastases through its potent proangiogenic activity. Raised circulating levels of VEGF have been identified in the early postoperative period (J. H. Harmey, unpublished observation). This may facilitate the growth and metastasis of circulating or intravasated dormant tumour cells.

Endotoxin induces angiogenesis in a dose-dependent fashion at biologically relevant doses[112]. Significant translocation of endotoxin into the peritoneal cavity and systemic circulation occurs following open surgery or air-insufflation laparoscopy[143]. It directly increases macrophage production of VEGF, a potent angiogenic factor regulating both vessel growth and permeability, an effect which also appears to extend to tumour cells[115,144]. Surgical trauma is known to increase leucocyte numbers and activity[145] and to promote tumour establishment and growth[146]. The authors' preliminary observations indicate that endotoxin introduced at the time of surgery may encourage the growth of metastases by suppression of host antitumour immune function and stimulation of proangiogenic factor release. The result is a permissive environment for tumour growth and angiogenesis.

It has been shown that the primary tumour produces circulating inhibitors of angiogenesis, called angiostatin and endostatin, which act specifically at the level of endothelial cell proliferation[121]. Within 5 days of removal of the primary tumour, angiostatin and endostatin disappear from the circulation and angiogenesis within dormant micrometastases becomes possible. This heralds a phase of rapid growth of previously dormant micrometastases[18]. Dormancy is normally maintained in tumour cells by means of a high replication rate and a correspondingly high apoptotic rate (*Fig. 2*). There is no change in the replication rate of neovascularized

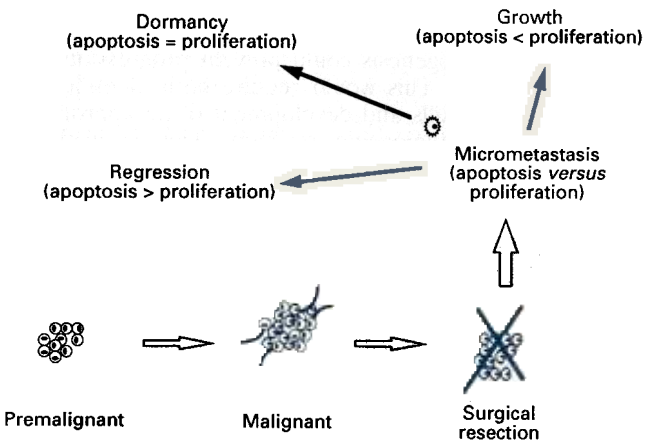

Fig. 2 Fate of micrometastases

metastases; however, the apoptotic rate is significantly decreased with resultant net metastatic growth[20].

Therapeutic opportunities (*Fig. 3*)

The aim of antiangiogenic treatment is to reduce and maintain tumours as small relatively dormant clusters of cells which have low metastatic potential, are more susceptible to cell-mediated immunological attack[3], and which may be more vulnerable to chemotherapy[147] and radiotherapy[148,149]. Tumour growth is associated with increased interstitial pressure due to leaky tumour vessels[150] in the absence of adequate lymphatic vessels[151]. This causes vascular compression and eventual central necrosis. As a result, tumours do not outgrow their blood supply, they compress it[152]. Antiangiogenic therapy paradoxically increases delivery of chemotherapy to a tumour[147] by reducing interstitial pressure[152]. Inhibition of angiogenesis results predominantly in a cytostatic, not a cytotoxic, effect[22,153–155], although a recent study suggests that antiangiogenic therapy may lead to tumour regression[156].

Successful antiangiogenic strategies have incorporated a wide range of antiangiogenic agents[121,157–163]. Prolonged courses of interferon α induce regression of human haemangiomatous disease[164]. Systemic administration of angiostatin induces regression of primary murine tumours

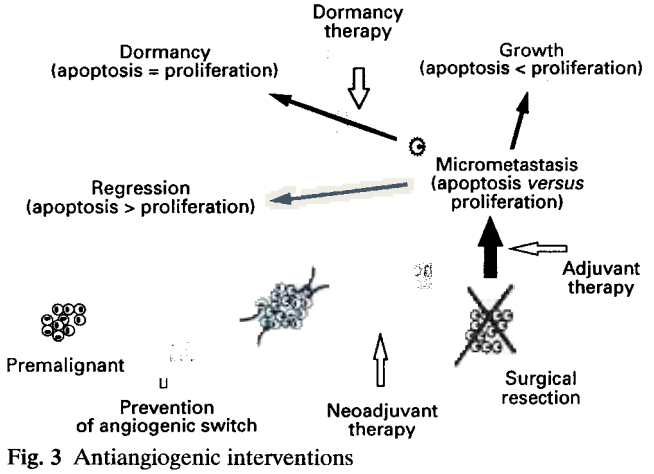

Fig. 3 Antiangiogenic interventions

of breast, colon and prostate[17]. Recombinant endostatin inhibits angiogenesis, the growth of metastases and the growth of primary tumours[123]. *In vivo* inhibition of angiogenesis using an animal model of pancreatic carcinoma has been shown to reduce tumour growth[165].

Mechanisms of action

Antiangiogenic therapies may act either directly, by specifically inhibiting endothelial cell proliferation, or indirectly, by altering the cytokine microenvironment which controls angiogenesis. Tumours consist of a 'two-compartment system' in which tumour cells and endothelial cells co-exist, and produce growth factors which have paracrine effects on each other[33]. Inhibition of endothelial cell growth reduces production of tumour-promoting substances and inhibits tumour expansion. Inadequate tumour vascularization may result in tumour cell apoptosis, a genetically programmed form of cell death[36]. Dormant micrometastases are biologically active with a rate of apoptosis equal to their rate of cell proliferation[122,166]. As a result, no net growth of the metastasis occurs. Antiangiogenic therapies promote and maintain perpetual dormancy by increasing the apoptotic rate within the metastasis, hence the appellation 'dormancy therapy'.

Direct antiangiogenic agents

Angiostatin and endostatin are the only direct inhibitors of angiogenesis identified to date, and both have been shown to reduce tumour growth[121,123]. Systemic administration of endostatin, which directly inhibits endothelial cell mitogenesis, has been suggested as an ideal 'dormancy therapy'[123]. More recently, it has been reported that repeated cycles of endostatin can prevent tumour recurrence, possibly through the induction of an antiangiogenic phenotype, without leading to the problems of drug resistance which characterize many chemotherapeutic agents[156]. Hanahan[167] has suggested that direct inhibitors of angiogenesis may be subdivided into three groups. First, pure angiogenesis inhibitors, which inhibit new vessel growth but have no effect on existing tumour vessels. Second, tumour vessel toxins, which damage existing tumour vasculature and, finally, dual action agents, which combine these two effects. It appears that endostatin is a member of the last group.

Indirect antiangiogenic agents

Indirectly acting antiangiogenic therapies influence the microenvironment which regulates tumour angiogenesis and so indirectly influence endothelial cell behaviour. The majority of antiangiogenic agents currently identified belong to this group. Each tumour has a unique complex microenvironment dictated both by tumour histology and site of implantation. Additionally, within any tumour, heterogeneity arises because of variations in stromal density, tumour differentiation, hypoxia and local cytokines, among other factors. As a result, each tumour is exposed to different proangiogenic and antiangiogenic stimuli which ultimately influence net angiogenesis. It is therefore unlikely that therapeutic manipulation of a single cytokine will be sufficient to regulate tumour angiogenesis adequately. Intertumour variability suggests that therapeutic regimens based on cytokine manipulation must be custom-designed for each particular tumour type.

Maximum clinical gain may potentially be achieved by a combination of antiangiogenic interventions which influence different aspects of angiogenesis. For example, the fumagillin analogue AGM-1470 reduces endothelial cell proliferation[157]. Interferon α interferes with endothelial cell migration[168] and IL-12 inhibits angiogenesis mediated by interferon γ[169]. In contrast, suramin inhibits bFGF receptor binding in addition to a number of direct effects on endothelial cells[170], while tamoxifen inhibits VEGF-mediated endothelial cell proliferation and migration[171]. Direct manipulation of endothelial cell proliferation combined with alteration of the cytokine microenvironment may prove more effective than either approach alone.

Multimodality therapy, in which antiangiogenic strategies are combined with chemotherapeutic agents or radiotherapy, is known to be more effective than monotherapy, probably because of the combined effects on both the tumour and vascular compartments[147–149,172].

Optimal methods and timing of administration of antiangiogenic therapies have yet to be determined. Unfocused interruption of angiogenesis may potentially interfere with wound healing, menstruation and embryogenesis. Inhibitors of endothelial-specific growth factors (e.g. VEGF) have a theoretical advantage over inhibition of growth factors with more widespread targets (e.g. bFGF) because undesired non-specific interruption of mitogenesis is avoided. However, it is likely that novel methods of site-specific drug delivery, developed to administer experimental proangiogenic therapies in the treatment of ischaemic heart disease, will also be applied to the delivery of antiangiogenic agents and may help to reduce unwanted systemic effects[56]. Substantial expression of VEGF receptors is predominantly limited to the tumour vasculature. Interference with this system using monoclonal antibodies, dominant negative receptor or antisense oligonucleotides against VEGF messenger RNA offer the potential of tumour-specific inhibition of angiogenesis and subsequent metastatic growth[173]. A similar approach could be pursued for other cytokines. Developments in the field of gene therapy[174] may be harnessed to produce long-term low-grade antiangiogenic substances which maintain micrometastases in a state of dormancy.

The dosing schedule for biological antiangiogenic agents is likely to differ significantly from that for pharmaceutical agents[18]. Clinical experience with interferon α in the treatment of life-threatening haemangiomas has shown that very prolonged courses of daily therapy at optimal doses are essential for vessel involution[175]. Timing of antiangiogenic therapy in relation to other therapeutic interventions also requires clarification. For example, excision of a primary tumour results in decreased levels of the inhibitory factors angiostatin and endostatin[18], and raised circulating levels of VEGF. In the postoperative setting, this is likely to facilitate the growth and metastasis of circulating or dormant tumour cells. Therefore, manipulation of tumour angiogenesis in the perioperative period provides a tempting therapeutic opportunity. The effect of impaired angiogenesis on wound healing, however, must be clarified. A balance between the neovascularization necessary for wound healing and inhibition of neoplastic microvessel formation must be achieved.

Variability in mechanisms and control of angiogenesis at different stages in the natural history of cancer may exist. A premalignant phase is recognized for many tumours. This is also a preangiogenic phase and development of an antiangiogenic strategy which prevents the onset of angiogenesis could prevent progression to a malignant state[176]. This would require both identification of premalignant cells and development of an appropriate prophylactic antiangiogenic regimen. This is likely to differ substantially from the therapeutic approach required for primary tumours or micrometastases. For example, VEGF messenger RNA expression develops late in the course of tumour progression, is not a feature of premalignancy[28] and its manipulation is, therefore, unlikely to be of benefit in this situation. Timing of prophylactic therapy will also be critical, as angiogenesis is probably not involved in the interim stage of micrometastasis following shedding from the primary tumour, when micrometastases are distributed in organs as either single cells or small clusters with ready access to a blood supply[20]. None the less, inhibition of angiogenesis is the most promising new therapy in the treatment of malignancy. Complete understanding of the factors regulating angiogenesis and the interactions between these factors should lead to valuable new interventions. These may be combined with surgery and other modalities to improve the treatment of many solid tumours.

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