ANTIANGIOGENIC THERAPY IN HUMAN GASTROINTESTINAL MALIGNANCIES

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SUMMARY

Angiogenesis is a crucial process involved in numerous physiological and pathophysiological settings. During the past three decades, the field of tumour associated angiogenesis has been the focus of a plethora of basic research projects and clinical studies. Tumour associated neovessels satisfying the increased demand of oxygen and nutrients in malignant tumours are now emerging as specific targets for novel antineoplastic agents. This article discusses the present data on antiangiogenic treatment of human gastrointestinal malignancies, including gastric, pancreatic, and colorectal adenocarcinoma, and outlines potential future perspectives, such as development of surrogate markers of angiogenesis.

PROCESS OF TUMOUR ANGIOGENESIS

Angiogenesis, the formation of new vessels from existing capillary beds, represents a central mechanism involved in many physiological and pathophysiological conditions, including embryonal development, wound healing, and chronic inflammation.[1] In malignant tumours, angiogenesis represents a prerequisite for tumour growth, as the high metabolism of tumour cells requires large amounts of oxygen and nutrients. Without angiogenesis, the growth of malignant tumours is limited to 1–2 mm in diameter, which corresponds to the maximum distance oxygen can diffuse.[2 3] Tumour vascularisation represents a *conditio sine qua non* for exponential tumour growth. Studies have indicated that the mitotic index of tumour cells decreases with increasing distance from the supplying microvessel.[1 3 4] Primary tumours as well as their metastases are dependent on tumour associated microvessels to establish an independent blood supply.[3 5] Enhanced vascularity allows tumours not only to expand in size but also facilitates haematogenous spreading by a higher probability of tumour angioinvasion. Once deposited in a distant organ, tumour metastases are dependent on building their own microvasculature.[6] For this reason, disruption of angiogenesis pathways provides a therapeutic perspective in the treatment of primary malignant human tumours as well as their metastases.

The concept that metastases are solely dependent on newly formed blood vessels in order to grow is opposed by observations made by Vermeulen and colleagues.[7] According to their findings, approximately one third of hepatic colon cancer metastases studied (n = 28) do not induce new vessels but rather use and preserve the existing abundant vascularisation of the liver parenchyma. This pattern, called ''replacement pattern'', differs from the so-called ''desmoplastic'' and ''pushing''-type of metastasis. In all three patterns of metastasis, overall vessel density was not significantly different. However, a much lower endothelial cell proliferation was encountered in the replacement-type of metastasis. These findings suggest that not counting vessels, but rather evaluating endothelial cell (EC) proliferation, might be the appropriate way for assessing angiogenesis, depending on the type of metastasis.[8]

The concept of human tumours being capable of secreting angiogenic factors was first introduced by Dr Judah Folkman in the 1970s, who is widely regarded as a pioneer of angiogenesis research.[9] Human microvascular angiogenesis is tightly controlled by a plethora of biochemical variables, including soluble factors (for example, cytokines, growth factors, enzymes modifying the extracellular matrix), as well as hypoxia and acidity (fig 1). Tumour angiogenesis is a mechanism by which the tumour responds to hypoxia and resistance to hypoxia may be attributed to overexpression of enzymes such as haemoxygenase-1 [10] and carbonic anhydrase.[11] Angiogenic factors are produced by a multitude of physiological cell populations,[12] including smooth muscle cells and pericytes,[13] endothelial cells,[14–16] and tumour cells, as well as epithelial cells from colorectal carcinoma.[17]

Vascular endothelial growth factor (VEGF) and many other angiogenic factors are endogenous ligands of receptors present on the EC surface, leading to initiation of intracellular signal transduction and gene transcription, eventually resulting in EC proliferation, active locomotion

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► Angiogenesis is a prerequisite for the growth of solid tumours.

(chemotaxis), and degradation of the extracellular matrix (ECM) by secretion of proteases, including matrix metalloproteinases (MMP).[18 19] However, MMP-2, -7, -9, and -12 were also shown to counteract angiogenesis through generation of potent endogenous angiogenesis inhibitors, including angiostatin, by proteolytic cleavage of plasminogen and specific collagen chains found in the ECM.[20]

Following rearrangement of the EC, a primary immature vascular network is formed which is subsequently refined by vessel maturation and consolidation by adjacent supporting cells, including smooth muscle cells and pericytes (fig 2).[21] Compared with physiological microvessels, tumour associated microvessels are fragile highly disorganised vessels of hetereogeneous diameters, which show less cellular support by scaffolding cells and extracellular matrices.[22 23] In addition, tumour microvessels exhibit defect vasomotor functions, often lacking a predilected direction of blood flow.[22] All of the above mentioned characteristics of tumour associated microvessels deserve consideration in the design of antiangiogenic strategies as disturbed blood flow and altered permeability potentially hamper effective drug delivery.[24 25]

Oncogenes and angiogenesis in solid tumours

Angiogenesis driven by solid tumours is believed to be dependent on genetic alterations that also account for features characteristic of malignant transformation, including resistance to apoptosis and deregulated mitogenesis. Genetic alterations responsible for the malignant behaviour of tumour cells include activation of various oncogenes, such as c-myc and HER-2, as well as inactivation or loss of tumour suppressor genes, including p53 and p16. Various oncogenes are known to be potent deregulators in the expression of angiogenic and angiostatic effector molecules by tumour cells. For example, activation of specific oncogenes (K-ras, H-ras, Her-2, c-fos, among others) is associated with enhanced expression of angiogenic mediators (for example, VEGF) by tumour cells. Likewise, these alterations are also involved in downregulation of important antiangiogenic mediators, such as thrombospondin. In addition to VEGF, activated ras oncogenes have also been implicated in the production of further angiogenic factors, including basic fibroblast growth factor (bFGF), transforming growth factor (TGF) family members, platelet derived growth factor (PDGF), insulin-like growth factor (IGF)-1, and others.[26]

Under physiological conditions, p53 gene product is maintained at low often undetectable protein levels owing to an extremely short half life. Under pathophysiological conditions, including DNA damage, activation of oncogenes, and hypoxia, p53 stabilisation occurs, which leads to higher levels of p53 expression.[27] Likewise, overexpression of p53 in colorectal carcinoma cells was associated with high microvascular densities in adjacent tissue areas.[28] Similar to these observations, a study by Liang *et al* reported that both K-ras mutation and mutant p53 overexpression status were significantly correlated with microvascular density in 114 colorectal carcinoma specimens.[29] Conflicting results were published in a study by Giatromanolaki *et al* where no correlation between p53 expression and the degree of tumour vascularity was observed in 106 colorectal cancer specimens.[30] These findings were supported by Aotake *et al*, who were

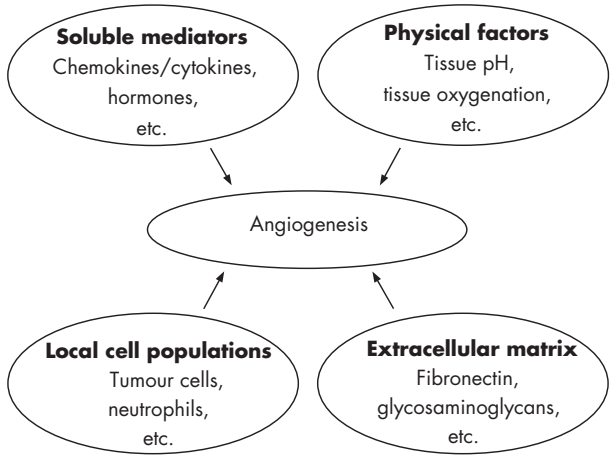

Figure 1 Factors controlling angiogenesis. The formation of new blood vessels from existing capillary beds is dependent on a multitude of physical, chemical, and biological factors. Soluble mediators, including vascular endothelial growth factor and other cytokines, as well as tissue pH and hypoxia, are important determinants regulating angiogenic activity. Likewise, both extracellular matrix and local cell populations have an impact on angiogenesis.

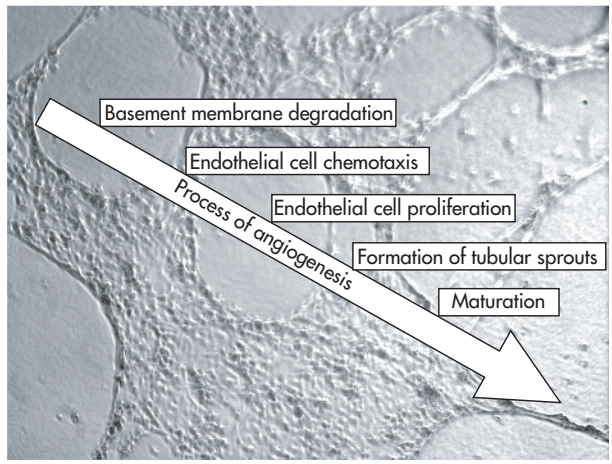

Figure 2 Angiogenesis: a multistep sequence. The process of angiogenesis is a sequence of events, some of which occur simultaneously. Proteolysis of the basement membrane is followed by directed locomotion of endothelial cells (chemotaxis). Endothelial cells begin to proliferate, forming initial tube-like structures (sprouting). The final event in this sequence is maturation of microvessels, which is supported by adjacent cells, including pericytes. Background picture: human intestinal microvascular endothelial cells forming tubular structures in an extracellular matrix (own observations).

► Tumour associated angiogenesis depends on a plethora of biochemical and physical determinants, including growth factors, tissue pH, and tissue oxygenation.

► Activation of oncogenes or loss of tumour suppressor genes is often associated with expression of angiogenic factors by tumour cells.

Table 1 Expression of angiogenic factors in colorectal carcinoma: association with clinical features

Factor	No of patients	Association	Reference
VEGF			
	52	+MVD, +metastasis, +proliferation index	Takahashi[64]
	100	+MVD, +Dukes grade	Nakasaki[66]
	152	−Differentiation, +lymphatic metastasis, +hepatic metastasis, +advanced stage	Ochiumi[94]
	163	+MVD, −prognosis, +hepatic metastasis	Kang[197]
	136	+MVD, −prognosis, +TP expression	Amaya[198]
	100	+MVD, −prognosis, +hepatic metastasis	Maeda[199]
	121	+Recurrence rate	Cascinu[200]
	259	+MVD, +liver metastasis, −mean survival	Harada[201]
	152	−Mean survival	Kaio[202]
PD-ECGF (thymidine phosphorylase)	163	+MVD, +tumour size, +advanced stage, +lymphatic metastasis, −prognosis	Takebayashi [203]
	86	−Lymphatic/haematogenous metastasis	Saito[204]
	32	−Mean survival	van Triest[205]
	148	−Prognosis	Matsumura[206]
HIF			
	149	+MVD, +advanced stage, +hepatic metastasis, +VEGF expression	Kuwai[101]
	87	+MVD, −mean survival, +COX-2 expression	Yoshimura[102]

VEGF, vascular endothelial growth factor; PD-ECGF, platelet derived endothelial cell growth factor; HIF, hypoxia inducible factor; MVD, microvascular density.
NS, no significant correlation; +, positively correlated; −, inversely correlated.

unable to describe an association between p53 activation status and extent of angiogenesis in colorectal carcinoma.[31] Similar observations have been published for gastric[32] and pancreatic adenocarcinoma (tables 1–3).[33]

A study addressing the question of whether oncogene activation or p53 status could be associated with the clinical response to antiangiogenic therapy was published recently. In a series of 295 patients, the expression status of the oncogenes k-ras and b-raf, as well as of the tumour suppressor gene p53 in colorectal cancer specimens did not correlate with the clinical response status to antiangiogenic therapy targeting VEGF (bevacizumab/Avastin).[34] Therefore, the relevance of these specific genetic alterations with respect to antiangiogenic therapy in human colorectal carcinoma remains unclear.

1.2. Angiogenesis independent tumour vascularisation

Alternative mechanisms by which microvascular endothelial cells may support growth of solid tumours include vasculogenesis, vascular co-option, vessel mosaicism, and vascular mimicry (fig 3).[35] The term postnatal vasculogenesis refers to a mechanism by which endothelial precursor cells derived from bone marrow incorporate into and contribute to physiological and pathological angiogenesis.[36] Accordingly, in contrast with angiogenesis, blood vessels will be newly formed and not be derived from existing microvessels. It has been hypothesised that solid tumours secreting VEGF, including colorectal carcinoma, might be able to recruit endothelial progenitor cells from bone marrow to initiate vasculogenesis.[37]

Table 2 Expression of angiogenic factors in gastric adenocarcinoma: association with clinical features

Factor	No of patients	Association	Reference
VEGF			
	163	+MVD, NS mean survival, +differentiation	Tanigawa[207]
	195	+Disease recurrence	Maeda[67]
	102	+Hepatic metastasis, -prognosis	Kimura[208]
	91	(VEGF-D): −prognosis	Juttner[209]
	120	−Prognosis	Maeda[210]
PD-ECGF (thymidine phosphorylase)	158	+MVD, +Size, +lymphatic metastasis, −prognosis	Takebayashi[211]
	120	+MVD, +hepatic metastasis	Maeda[212]
	33	(Tissue activity): + venous invasion	Nakata[213]
	126	+Response to 5-FU chemotherapy, +prognosis	Saito[214]
	148	+MVD, −prognosis, +proliferation index	Kikuyama[215]
	102	+Hepatic metastasis, −prognosis	Kimura[208]
	116	−Prognosis, +proliferation index	Konno[216]
	116	+Haematogenous metastasis, +recurrence	Konno[217]
Ang-2			
	85	+Advanced disease, −mean survival	Etoh[93]
	72	+VEGF expression, +advanced disease	Sun[218]

VEGF, vascular endothelial growth factor; PD-ECGF, platelet derived endothelial cell growth factor; Ang-2, angiopoietin 2; MVD, microvascular density; 5-FU, 5-fluorouracil.
NS, no significant correlation; +, positively correlated; −, inversely correlated.

Table 3 Expression of angiogenic factors in pancreatic adenocarcinoma: association with clinical features

Factor	No of patients	Association	Reference
VEGF			
	22	NS MVD, NS mean survival, NS recurrence	Ellis[219]
	40	+MVD, −mean survival, +MVD	Ikeda[70]
	70	+MVD, +recurrence, −mean survival	Niedergethmann[220]
	55	+MVD, −mean survival	Kuwahara[79]
	124	+MVD	Khorana[221]
	50	+MVD, −mean survival	Couvelard[105]
FGF			
	55	NS MVD, −mean survival	Kuwahara[79]
	104	+MVD, +hepatic metastasis, +recurrence	Fujioka[222]
	20	+Proliferation index	Yamazaki[223]
PD-ECGF (thymidine phosphorylase)	104	+MVD, −mean survival, +recurrence, +hepatic metastasis	Fujioka[222]
	50	+MVD, −mean survival	Fujimoto[69]
	40	−Mean survival	Ikeda[70]
	55	NS MVD, NS mean survival	Kuwahara[79]
	124	NS MVD	Khorana[221]
HIF			
	49	+MVD, +tumour size, +advanced stage, +proliferation index	Kitada[224]
	55	+MVD, +metastasis, +VEGF expression, −prognosis	Shibaji[104]
	50	+MVD	Couvelard[105]

VEGF, vascular endothelial growth factor; PD-ECGF, platelet derived endothelial cell growth factor; HIF, hypoxia inducible factor; MVD, microvascular density. NS, no significant correlation; +, positively correlated; −, inversely correlated.

Vascular co-option, recruitment of pre-existent vessels by tumours, was first described by Holash and colleagues in 1999.[38] This mechanism was initially observed in experimental gliomas, which arise in one of the most densely vascularised organs, the brain. Contrary to the view that angiogenesis is an immediate tissue response required for initial tumour growth, these tumours were shown to initially induce apoptotic vessel regression, resulting in a secondary

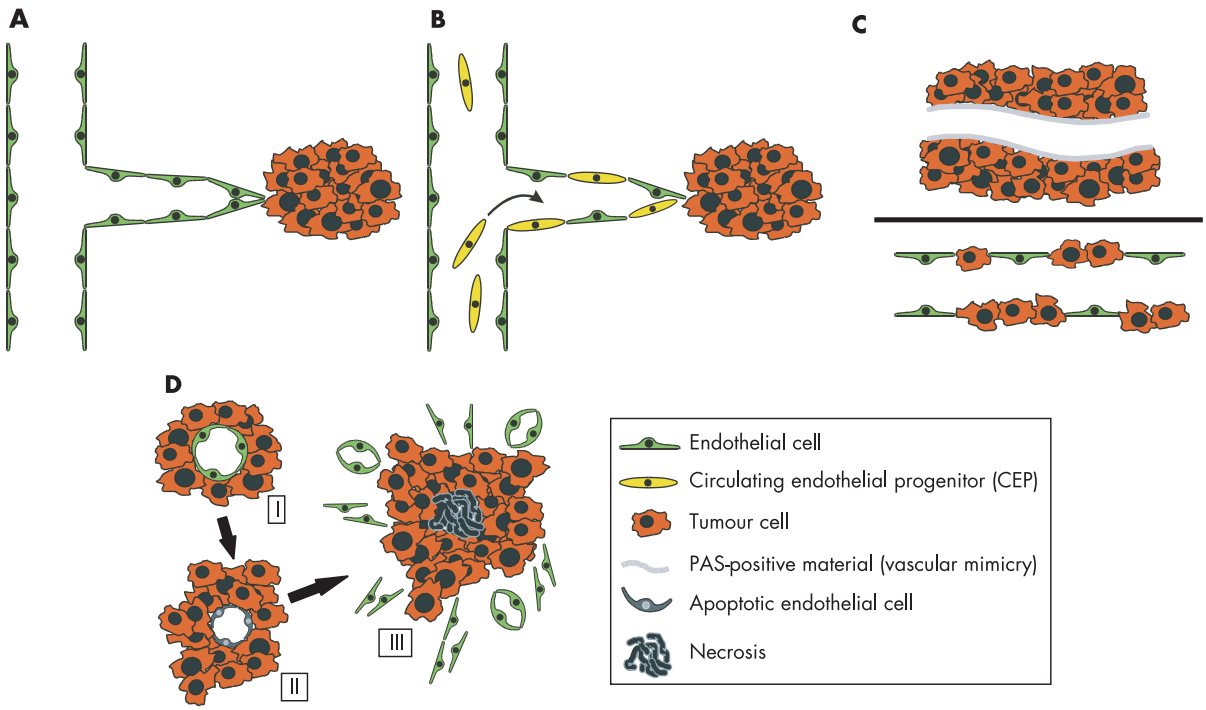

Figure 3 Mechanisms of tumour vascularisation. (A) Classical angiogenesis has widely been described as the formation of new blood vessels from existing capillary beds. Endothelial cells stimulated by angiogenic mediators start to proliferate, forming directed microvessels. (B) Vasculogenesis by recruitment of circulating endothelial progenitor cells (CEP) has been proposed as an additional mechanism of neoangiogenesis. Circulating CEP settle and differentiate into endothelial cells to establish new microvessels. (C) Vascular mimicry (upper panel) describes small perfused channels forming within clusters of tumour cells. Typically, these functional channels are lined with periodic acid-Schiff (PAS) positive material. The term vessel mosaicism (lower panel) refers to microvessels where the continuous endothelial layer is interrupted by interspersed tumour cells. (D) The term vascular co-option refers to a mechanism by which tumour cells surround supporting microvessels (I), causing endothelial cell apoptosis. Subsequent hypoxia leads to upregulation of angiogenic mediators by tumour cells, which results in strong angiogenesis, mainly at the tumour periphery (III). Modified from Auguste and colleagues.[35]

- Alternative mechanisms of tumour vascularisation include vasculogenesis, vascular mimicry, vessel mosaicism, and vascular co-option.

avascular tumour. In later stages, a robust proangiogenic response at the tumour margin is observed, resulting in tumour rescue.[38] Correspondingly, tumours arising in densely vascularised organs will initially be able to grow without the need of an angiogenic switch to take place. Therefore, this initial form of blood supply will not be affected by angiogenesis inhibition.[39]

In recent years, it has been hypothesised that tumour cells themselves are able to participate in the formation of functional vessel-like channels. Vascular mosaicism, a mechanism whereby the tumour cells themselves line the vessels, was reported to occur in up to 15% of vessels in a colonic carcinoma implantation model.[40]

The concept of vasculogenic mimicry was first described for aggressive uveal melanoma described by Maniotis *et al*. This tumour was shown to contain a network of channels interconnected by loops. Transmission electron microscopy indicated that the channels are lined by a basal lamina-like layer. Inside the channel lumen, red blood cells are observed. Functional assays have indicated that these channels might contribute to tumour perfusion, as shown by experimental perfusion using fluorescent dyes.[41]

All of the aforementioned alternative vascularisation models might have important consequences for the development of antiangiogenesis agents in human gastrointestinal tumours, but clinical data are lacking to date.

Recent research has focused mainly on inhibition of single prominent proangiogenic factors and selected pathways. This review reports on a selection of established and well recognised proangiogenic factors, although many more are currently under intensive scientific and clinical investigation.

ANGIOGENESIS RESEARCH: ANIMAL MODELS VERSUS HUMAN PRIMARY CELL CULTURE

Most of what is known about angiogenesis is derived from research on animals (for example, tumour implantation models using immunocompromised SCID mice injected with human colon cancer cells). The large gap between rodent vascular biology and human disease is one major point of criticism in the assessment of clinical antiangiogenesis studies. Many therapeutic strategies obtained from rodent angiogenesis models have proved disappointing in the treatment of human disease.[42 43] This is most likely caused by the marked differences in human and rodent vascular biology, as well as by endothelial heterogeneity in human in vitro EC models.[44] Consequently, clinical angiogenesis research demands simulation of human intestinal vascular pathology in vitro to obtain results resembling human in vivo vascular characteristics.

In 2000, St Croix and colleagues published a study on specific gene transcription patterns of EC isolated from human colorectal tumours compared with EC from human normal colonic mucosa. Using this approach, 79 genes were differentially expressed, including 46 that were selectively upregulated in tumour associated EC. Some of the detected genes encode ECM proteins but the majority of genes are of unknown function.[45]

With primary cultures of human intestinal microvascular EC in vitro,[46] experiments can be performed resembling the in vivo features of the human intestinal microvasculature as closely as possible.

Multiple experimental approaches to quantify and characterise angiogenesis have been tested and reported. Angiogenesis, a multistep process of interdigitating cellular activities, is composed of cellular locomotion, as assessed by stress fibre assembly and chemotaxis assays, as well as proliferative responses, which can be measured in vitro by cellular uptake of tritiated thymidine (^{3}H-thymidine, reflecting DNA synthesis) or catalysation of methylthiazolyldiphenyl-tetrazolium bromide oxidation (reflecting mitochondrial metabolism).[47] In addition, a recent investigation has shown evidence for a role of directed apoptosis in the formation of new blood vessels.[48] Direct readouts for in vitro angiogenesis are provided using a three dimensional extracellular matrix (Matrigel) enabling cultured EC to form tube-like structures. In vivo angiogenesis assays are represented mainly by subcutaneous implantation of cast Matrigel plugs and post mortem histological analysis or the chicken chorioallantoic membrane assay.[49]

ANGIOGENIC FACTORS

Vascular endothelial growth factor (VEGF)

VEGF is regarded as one of the most important angiogenic factors in health as well as in disease. The VEGF family is comprised of six molecules termed VEGF-A, B, C, D, and E, and placenta growth factor.[50] VEGF-A exists in five isoforms (121, 145, 165, 189, and 205 amino acids),[51] the largest two of which are membrane bound. VEGF family members act on EC via VEGF receptors expressed on the EC surface, inducing EC proliferation, synthesis of proteolytic enzymes,[52 53] and chemotaxis.[54] Further effects of VEGF-A include antiapoptotic functions, as well as vasodilatation[55] and potent escalation of vascular permeability, allowing for plasma proteins to diffuse into the perivascular interstitium to constitute a reticular network along which EC migrate to form premature vessels.[56] EC stimulation with VEGF also results in secretion of proteases involved in extracellular matrix degradation, a mechanism required for angiogenesis. VEGF-A (165) seems to be the most predominant isoform secreted by human tumours.[57] Factors modulating VEGF expression were shown to include decreasing tissue pH, hypoxia, and various growth factors, among others.[58] Activation of all three characterised VEGF receptors (VEGFR-1 (flt-1), -2 (kdr/flk-1), -3 (flt-4)) expressed on the surface of EC and some tumour cell populations results in intracellular tyrosine kinase activity, mediating a potent mitogenic stimulus.[59 60] More specifically, VEGFR-1 was found to communicate EC migration, while stimulation of VEGFR-2 leads to an increase in vascular permeability and EC proliferation.[61]

Expression of VEGF-A is highly upregulated in human colorectal adenocarcinoma,[62] and high expression of this cytokine has been linked to poor prognosis and a high propensity of metastatic spreading in these patients.[63 64] Additional evidence for a pivotal role of VEGF-A in angiogenesis of colorectal adenocarcinoma comes from observations indicating increasing expression from non-neoplastic to malignant colonic mucosa.[65] Additionally, microscopic vessel counts were found to tightly correlate with tumour expression of VEGF in colorectal adenocarcinoma.[66] Further study results suggest that VEGF expression levels and vessel counts may be used as prognostic markers

for recurrence and metastasis in colon cancer patients.[64] Similar observations have been made for expression of VEGF-A in gastric[67 68] and pancreatic adenocarcinoma.[69 70]

Fibroblast growth factors

Fibroblast growth factors (FGFs) constitute a large family with no less than 20 related molecules with a wide spectrum of biological functions, some of them exerting potent induction of angiogenesis in vitro and in in vivo models. Among these, the acidic FGF (aFGF, FGF-1) and basic FGF (bFGF, FGF-2) have been investigated most profoundly. As known for VEGF family members, the cellular activities of FGF are mediated by FGF receptor (FGFR1–4) associated intracellular tyrosine kinase activity. In correspondence to what is known about the biological functions of VEGF, FGFs were found to be potent inducers of EC proliferation and migration, as well as EC tubulogenesis.[71 72] Numerous additional functions of the FGF family have been associated with tissue repair and tumour progression. Interestingly, FGF-2 concentrations were found to be elevated in the urine of patients suffering from various malignancies.[73 74] In colorectal cancer, bFGF plasma levels were shown to correspond to advanced tumour stages, as well as resistance of tumours to chemotherapy.[75–77] Only limited data are available regarding expression of FGFs in gastric and pancreatic carcinoma. Initial results obtained by Tanimoto *et al* have indicated elevated expression of bFGF mRNA in 55% of gastric carcinoma tissues compared with control tissue.[78] In pancreatic carcinoma, immunostaining results have shown that FGF-2 was detectable in 60.9% of tumour specimens. Furthermore, high expression levels of FGF-2 were significantly associated with shorter survival times in these patients.[79]

Platelet derived endothelial cell growth factor

Platelet derived endothelial cell growth factor (PD-ECGF) is a thymidine phosphorylase acting as a powerful chemoattractant on EC,[80] which exerts marked angiogenic responses in rodent tumour models.[81] In addition to its functions as a secreted growth factor, PD-ECGF is involved intracellularly in the metabolism of pyrimidine nucleosides and 5-fluorouracil.[82] Expression of PD-ECGF has been shown in tumour cells, stromal cells, and infiltrating mononuclear cells.[83] It has been hypothesised that human colorectal tumours showing low VEGF expression are more dependent on PD-ECGF as the major proangiogenic factor.[83] As for VEGF-A, expression of PD-ECGF by tumours has been linked to poor prognosis in pancreatic[70] and gastric[84] cancer. Recent studies have suggested that tumour PD-ECGF expression might serve as a prognostic marker for the outcome and response to chemotherapy in patients with colorectal cancer,[85 86] especially when assessed at the edge of the invading tumour.[87]

Angiopoietins

The angiopoietins Ang-1, -2, -3, and -4 are agonistic ligands of the Tie-2 tyrosine kinase receptor generally expressed on EC surfaces.[88] The Tie-2 receptor is activated by Ang-1 and Ang-4 while Ang-2 and -3 were shown to counteract Ang-1 induced Tie-2 activation.[88 89] The angiopoietin effects therefore result from the finely tuned balance of Ang-1 to -4 family members. Interestingly, the effects of Ang-2 seem to be tightly dependent on the concomitant presence of the major angiogenic factor VEGF. In the absence of VEGF, Ang-2 was shown to induce vessel regression whereas in the presence of VEGF, Ang-2 is believed to support the angiogenic response of the microvessel.[89] Ang-2 is commonly overexpressed in colorectal adenocarcinoma while Ang-1 was rarely found to be expressed.[90 91] In addition, colon cancer cells stably transfected to overexpress Ang-2 and implanted into immunocompromised mice displayed markedly enhanced growth kinetics compared with untransfected control cells.[92] A high tumour expression of Ang-2 was correlated with advanced tumour stages and shorter survival in gastric[93] and colorectal[94 95] cancer patients. In addition, Ang-2 expression was found to be markedly elevated in pancreatic carcinoma tissue samples,[96] but data on its impact on the outcome and prognosis are lacking.

Hypoxia inducible factor 1

Hypoxia inducible factor (HIF) 1 is known to play a central role in tissue responses to hypoxia. HIF-1 is a heterodimeric protein consisting of two subunits called HIF-1α and 1β. Under normoxic conditions, HIF-1α is rapidly degraded in a proteasome dependent pathway. In human tumour cells, HIF-1α degradation was shown to be tightly dependent on the activation status of the tumour suppressor gene, p53. Anoxic conditions were reported to maximise p53 activation, which leads to an increase in HIF-1α degradation.[27] In hypoxia, however, HIF-1α degradation is markedly diminished, resulting in the formation of stable HIF-1 heterodimers and eventually leading to activation of specific genes whose products act to increase the oxygen concentration in the tissue (for example, VEGF and haeme oxygenase, among others).[97] Additionally, hypoxia independent upregulation of HIF-1 was described, occurring as a downstream event of growth factor signalling (for example, epidermal growth factor receptor activation).[98]

In in vitro models using colorectal carcinoma cells, expression of HIF-1 was linked to increased tumour vascularisation and higher invasiveness.[99] Data from a study using human gastric cancer cells implanted in mice have indicated that inhibition of HIF-1α leads to a reduction in tumour associated angiogenesis and vessel maturation, accompanied by impairment of tumour growth.[100] Overexpression of HIF-1 in human colorectal adenocarcinoma has been demonstrated to correlate with tumour VEGF expression and advanced tumour stages.[101 102] Recent data have shown that tissue expression of HIF-1 in colorectal cancer specimens appears to be linked to that of lactate dehydrogenase, isoform 5, a response marker for tissue hypoxia and anaerobic glycolysis. Both of the above factors were shown to be associated with an aggressive cancer phenotype in patients with colorectal adenocarcinoma.[103] In pancreatic adenocarcinoma, expression of HIF-1α was shown to correlate with histological markers of angiogenesis and prognosis.[104 105]

Angiogenic chemokines

Chemokines are small (8–12 kDa) secreted proteins serving a wide array of receptor dependent immune functions.[106] Chemokines displaying the ELR (Glu-Leu-Arg) amino acid motif (ELR^+ chemokines) have been shown to have direct angiogenic effects on human EC in vitro and in vivo, with interleukin 8 (IL-8) being the most extensively studied angiogenic chemokine.[107] IL-8 (also termed CXCL8) shows constitutive and regulated expression in a broad range of cells, including tumour infiltrating mononuclear cells, several human tumour cell lines, and EC. Proinflammatory regulation of IL-8 is mediated by the transcription factor nuclear

factor κB.[108–110] IL-8 exerts its biological activities on effector cells in a paracrine and autocrine fashion. The cellular effects of IL-8 are mediated by ligation of its cognate receptors, CXCR1 and CXCR2 expressed on target cells, including human EC.[111] The chemokine receptor CXCR2 promiscuously binds all known members of the angiogenic ELR$^+$ CXC chemokine family, including IL-8, the growth regulated oncogene family members (GRO-α, -β, and -γ), NAP-2, GCP-2, and ENA-78 with high affinity. In contrast, CXCR1 specifically binds only IL-8 and GCP-2. Initially identified as a major proinflammatory cytokine in various inflammatory disorders, there is growing evidence that IL-8 exerts potent angiogenic effects in human malignant tumours, including colorectal adenocarcinoma. In addition to its direct action on endothelial cells, *in vitro* angiogenesis induced by exogenous IL-8 was frequently accompanied by inflammatory bystander cells, pointing towards additional angiogenic mechanisms by IL-8-mediated release of secondary angiogenic mediators.[112] Malignant colonic epithelial cells derived from colorectal adenocarcinoma are known to secrete IL-8 in a regulated fashion in vitro.[108] Strategies blocking the angiogenic activity of IL-8 have proven to be effective in inhibiting angiogenesis in human tumours in murine models.[113 114] Notably, IL-8 is highly expressed in hyperplastic mucosa adjacent to colon cancer, supporting an indirect angiogenic effect of colon cancer cells.[115] In addition, IL-8 seems to be involved in the development of distant metastases from colorectal cancer.[116] Data from our group have indicated that primary human intestinal microvascular endothelial cells derived from human gut exclusively express CXCR2, whereas CXCR1 does not appear to be expressed.[117] For this reason, human intestinal microvascular endothelial cells appear to be responsive to an array of angiogenic chemokines.

In human gastric cancer models, malignant gastric epithelial cells stably transfected to overexpress IL-8 show increased angiogenesis and tumorigenesis in nude mice.[118] Similar experimental observations were made in human pancreatic adenocarcinoma cells orthotopically transplanted into nude mice.[119]

An additional potential CXC chemokine receptor expressed on colonic microvascular endothelial cells is the Duffy antigen receptor for chemokines (DARC). DARC has been shown to non-selectively bind various angiogenic ELR$^+$ CXC chemokines, including IL-8.[120] Although DARC is highly expressed on human colonic mucosal microvessels (Heidemann, unpublished data), its signalling properties are unclear as it apparently does not couple to G proteins, and binding of chemokines to DARC does not evoke any intracellular signal in vitro.[121 122] It has been suggested that DARC might act as a decoy and scavenger receptor binding excess chemokines, potentially sustaining chemokine action in a buffering fashion.[123] To date, expression of DARC in tumour microvessels and its role in chemokine driven angiogenesis remains unclear.

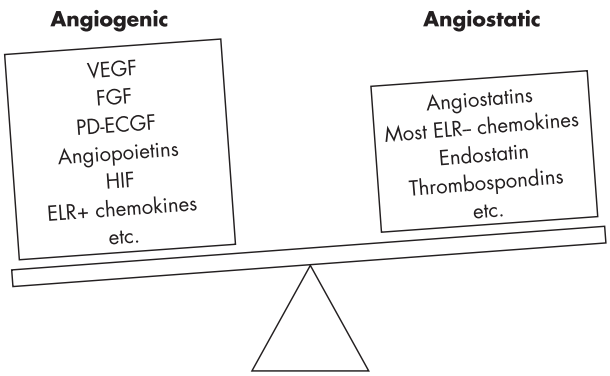

Figure 4 Endogenous factors regulate angiogenesis. The extent of angiogenesis is controlled by the net balance of local endogenous angiogenic and angiostatic factors. An increase in angiogenic factors, as well as a decrease in angiostatic factors, will result in activated angiogenesis. VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; PD-ECGF, platelet derived endothelial cell growth factor; HIF, hypoxia inducible factor.

Other angiogenic factors

A multitude of additional secreted mediator molecules have been proven to have an impact on in vitro and in vivo angiogenic EC responses. In many cases, the underlying mechanisms of action are mediated by direct effects on EC, as for interferons α, β, γ, TGF-α and β, tumour necrosis factor α (TNF-α), thrombospondin, and many more (fig 4).[111] Alternatively, growth factors indirectly act on EC by secondary modulation of VEGF expression, as has been shown for various angiogenic mediators, including hepatocyte growth factor,[124] epidermal growth factor,[125] IGF-1,[126] IL-1 family members,[127] PDGF, TGF-β1,[128] and prostaglandin E2,[129] the production of which is tightly dependent on cyclooxygenase (COX)-2 activity, a key enzyme in mucosal microvascular homeostasis.[130 131]

ANTIANGIOGENIC THERAPY

Several antiangiogenic therapy concepts have resulted in preclinical models as well as clinical trials in human colorectal cancer disease. Clinical phase I and II trials of single antiangiogenic agents in human disease have been cancelled due to lack of efficacy. This is in part explainable by the fact that data obtained from rodent angiogenesis models are not translatable into hypotheses on human gastrointestinal angiogenesis without restriction. Compared with human vascular biology, rodent models feature a different set of angiogenic factors fine tuning the balance of microvessel growth and regression. Because of that, only a fraction of clinical trials in human disease can feasibly mimic the effectiveness of preclinical animal experiments. Angiogenesis in both rodents and humans is dependent on a multitude of biological and physical factors, which emphasises that effective antiangiogenic therapy regimens will have to address multiple mechanisms of antiangiogenesis.

The fact that it is quite difficult to determine whether the drug is really hitting the target molecule might be another explanation for the lack of efficacy demonstrated in several antiangiogenesis trials. Therefore, clinical antiangiogenesis trials should be designed in order to verify the direct effects on local endothelial cells (for example, by obtaining sequential biopsies of the tumour). In addition, many studies to date have recruited unselected patient populations. With respect to the above mentioned ''replacement pattern'' of metastasis, it can be speculated that antiangiogenic compounds will be ineffective owing to the low fraction of proliferating EC in this patient subset. Both of the above problems should be considered in the design of clinical antiangiogenesis trials.

It has been shown that effective antiangiogenic therapy leads to an increase in apoptosis in tumour cells, while tumour cell proliferation itself remains widely unaffected.

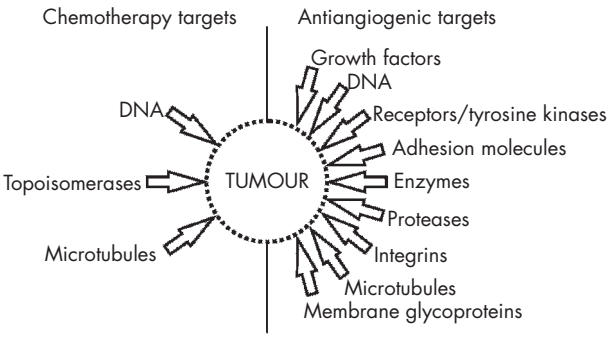

Figure 5 Diversity of targets in conventional chemotherapy and antiangiogenic therapy of human tumours. The multitude of potential antiangiogenic cellular targets permits more diverse mechanisms of action compared with conventional chemotherapy of tumours. Modified from Kerbel and colleagues.[196]

This balance of proliferative and apoptotic cell fractions is believed to constitute what is clinically referred to as tumour dormancy.[132] The multitude of angiogenic effector molecules brings about many potential escape mechanisms. On the other hand, these factors offer an abundance of potential targets for antiangiogenic therapy compared with conventional cytotoxic chemotherapy (fig 5). Therefore, combinations of multiple antiangiogenic agents with established cytotoxic chemotherapy regimens will likely emerge in future antiangiogenesis trials. As VEGF is regarded as the most prominent proangiogenic factor in human colorectal cancer, many of the therapeutic approaches are focusing on interruption of the biological activity of VEGF by means of neutralising circulating VEGF, competitive binding to its receptors, or inhibition of VEGF associated intracellular proangiogenic signalling cascades, including VEGFR associated tyrosine kinases.[133]

Neutralisation of VEGF biological activity

Bevacizumab (Avastin; Genentech, South San Francisco, USA) is a neutralising recombinant humanised monoclonal antibody against human VEGF that has completed phase III clinical trials and has obtained FDA approval for firstline treatment in combination with intravenous 5-fluorouracil (5-FU) based chemotherapy in February 2004. In a study conducted by Hurwitz *et al*, a total of 813 naive patients with metastatic colorectal cancer were randomly assigned to conventional intravenous IFL (irinotecan/5-FU/leucovorin) chemotherapy versus IFL plus bevacizumab. The results were indicative of a significantly prolonged total survival (20.3 *v* 15.6 months; $p<0.001$), progression free survival (10.6 *v* 6.2 months; $p<0.001$), and higher response rates in the bevacizumab group (44.8% *v* 34.8%; $p = 0.004$), respectively.[134] Recent data obtained from a phase II study indicate that addition of bevacizumab to chemotherapy regimens containing bolus 5-FU/leucovorin in patients who did not qualify for irinotecan based chemotherapy results in significant patient benefit, including prolonged progression free survival.[135] Consequently, bevacizumab in combination with a 5-FU based chemotherapy regimen has recently been approved in the USA and several European countries for firstline treatment of metastasised colorectal adenocarcinoma. The predominant side effects of bevacizumab are likely explained by direct effects on vascular homeostasis, including arterial thromboembolic events, gastrointestinal perforations, and impairment of wound healing, along with hypertension, proteinuria, and epistaxis.[136] In human rectal cancer, bevacizumab therapy is directly correlated with a decrease in tumour perfusion, microvascular density, and vascular volume, as well as with an increase in the fraction of vessels with pericyte coverage in rectal carcinoma patients, as assessed by pre- and post-treatment tumour tissue analysis.[137]

Further research projects are currently focusing on VEGF-Trap, a potent antiangiogenic soluble recombinant decoy protein constructed from VEGFR1 and VEGFR2 binding domains fused to a human immunoglobulin G1 constant region peptide.[138] Its biological affinity for VEGF is reported to be significantly higher than that of bevacizumab.[139] In preclinical rodent models, VEGF-Trap was shown to possess potent antiangiogenic efficacy[140–142] and is currently being studied in phase I clinical trials in patients with advanced stage solid malignancies, including colorectal adenocarcinoma. IMC-1C11, a chimeric antiVEGFR2/KDR antibody, is a further biological designed to block VEGF induced angiogenesis in human tumours. However, clinical testing of this antibody has not been completed.[143]

Small molecule compounds: inhibitors of tyrosine kinases and matrix metalloproteinases

Inhibition of angiogenesis has been the focus of many commercial research groups worldwide. Novel therapeutic agents lacking the severe side effects of conventional cytotoxic chemotherapy represent a welcome addition to established therapy regimens. Many antiangiogenic molecular compounds are currently under intensive investigation, most acting on receptor associated intracellular tyrosine kinase activities as one of the possible molecular targets (fig 6). Novel compounds showing favourable preclinical data were however lacking clinical efficacy in many cases. For example, SU5416 (Semaxanib), a potent and selective inhibitor of VEGFR2 tyrosine kinase activity, has failed to show efficacy in the treatment of human advanced colorectal cancer.[144 145] Additional phase III failures were observed for antiangiogenic MMP inhibitors (BB2516 (Marimastat), AG3340, Bay-12-9566)[146] in the treatment of various human solid tumours, including pancreatic adenocarcinoma. Another potent angiogenesis inhibitor, SU6668, a chemical compound acting on a variety of tyrosine kinase activities associated with VEGF, PD-ECGF, and FGF receptor activation, has undergone preclinical assessment and early clinical studies. Due to dose related toxicity, SU6668 had to be withdrawn from further testing.[147]

Further tyrosine kinase inhibitors include PTK787/ZK222584 (Vatalanib) and PKI 166, both of which have been shown to be effective in preclinical angiogenesis models in gastrointestinal tumours. PTK787/ZK222584 is currently being evaluated in phase II clinical trials in the treatment of gastrointestinal tumours, showing favourable data towards a biological response in tumour patients, along with a low occurrence rate of side effects.[148 149]

Additional VEGF-tyrosine kinase inhibiting small molecules are under clinical investigation as potential antiangiogenic compounds in various human solid tumours, including

► Bevacizumab, in adjunct with conventional chemotherapy, has proven to be effective in the first-line treatment of metastasized colorectal carcinoma.

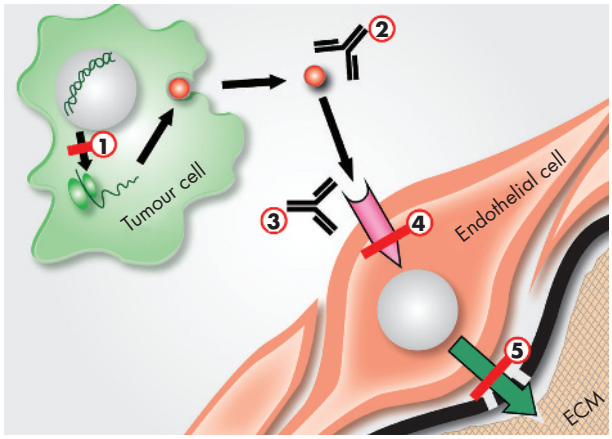

Figure 6 Subcellular localisation of antiangiogenic target molecules. The process of tumour associated angiogenesis can potentially be interrupted at various stages in the multistep sequence of tumour associated microvascular angiogenesis. (1) Some tumour cells show enhanced transcription of angiogenic mediators (red dot) (for example, vascular endothelial growth factor (VEGF)). Chemically stabilised phosphorothioate antisense oligonucleotides can be used to interfere with the transcription of angiogenic mediators. (2) Angiogenic mediators can be biologically bound by immunoreactive antibodies (for example, neutralising anti-VEGF antibodies) or other neutralising peptides (for example, VEGF-Trap). (3) Similarly, endothelial cell associated receptors specific for the angiogenic mediator (for example, VEGFR2) can be blocked by specifically designed antibodies eliciting competitive binding. (4) Receptor associated angiogenic signalling is often mediated by tyrosine kinases coupled to the angiogenic receptor (for example, VEGFR2 associated tyrosine kinases). Chemical compounds engineered to interfere with the enzymatic activity of these kinases (for example, Vatalanib) can specifically inhibit cytokine mediated endothelial activation. (5) Activated endothelial cells secrete proteases that potently disintegrate the endothelium associated basal lamina (black bold line) and the underlying extracellular matrix (ECM). Chemical compounds designed to block these enzymatic activities (for example, matrix metalloproteinase inhibitors) inhibit this last step in the sequence of tumour associated microvascular angiogenesis.

ZD6474 and AZD2171.[150] Other agents potentially effective in the antiangiogenic treatment of human colorectal cancer include BAY 43-9006 (Sorafenib),[151] a novel signal transduction inhibitor designed to disrupt angiogenesis through inhibition of the Raf/MEK/ERK pathway, and VEGFR2 associated tyrosine kinase activities, as well as SU-11248 (Sunitinib), an orally active inhibitor of multiple angiogenic tyrosine kinase signalling pathways.[152] All of the latter have undergone early clinical studies in the treatment of gastrointestinal cancer. Current studies actively recruiting gastrointestinal cancer patients are shown in table 4.

Other antiangiogenic treatment strategies

Future antiangiogenic approaches potentially include the use of stable nuclease resistant phosphorothioate antisense oligonucleotides aiming to reduce expression levels of tumour associated proangiogenic targets, including VEGFR-mRNA. Inhibition of VEGFR-mRNA was effective in reducing the peritoneal dissemination of experimental gastric cancer models in nude mice.[153] Other studies have indicated that chemically stabilised ribozymes designed to specifically cleave VEGFR coding mRNA could be effective in the inhibition of tumour growth and metastatic activity in rodent xenograft models of metastatic human colorectal cancer.[154 155] In addition, preclinical data have hinted at the effectiveness of small antagonistic peptides in antiangiogenic therapy, including integrin $\alpha_5\beta_1$ inhibitory peptides,[156] which were reported to be active in the reduction of hepatic metastasis, resulting in improved survival in a rodent colorectal cancer model.[157 158]

Inhibition of COX-2 activity has been shown to be effective in a wide variety of preclinical models of solid human tumours, including colorectal carcinoma.[131] COX-2 inhibition was shown to have antiangiogenic effects by downregulation of prostaglandin E2, which in turn is known to potently enhance expression of the angiogenic mediators VEGF and bFGF in an array of target cells, including endothelial cells and colorectal carcinoma epithelial cells.[159] Expression of COX-2 in colorectal cancer specimens was tightly linked to higher microvessel densities and increased VEGF gene expression.[160] Clinical studies justifying the use of such agents are lacking so far. As colorectal cancer patients are at increased risk of thrombotic events and COX-2 inhibitors have been linked to increased risk of thrombosis, the potential for an additive risk has to be considered.[161]

Thalidomide, a hypnotic drug, possesses anti-inflammatory and antiangiogenic properties, potentially by downregulation of TNF-α expression. Thalidomide has been used in combination with palliative chemotherapy regimens using irinotecan (CPT-11) in colorectal cancer patients. Apart from its ability to ameliorate irinotecan induced side effects, especially nausea and diarrhoea,[162] thalidomide is undergoing clinical testing[163 164] due to its antiangiogenic effects on colorectal carcinoma in preclinical models.[165]

Angiogenesis is tightly dependent on the viability of involved endothelial cells. Few studies have investigated the use of antiangiogenic compounds in combination with irradiation therapy. Preliminary evidence supports the concept that irradiation combined with the orally bioavailable VEGF tyrosine kinase inhibitor PTK787/ZK222584 exerts synergistic antiangiogenic effects on VEGF induced endothelial cell proliferation in vitro and a xenograft tumour model of human colorectal carcinoma.[166] Similar observations have been made for a variety of human solid tumour implantation models in combination with neutralising anti-VEGF monoclonal antibodies.[167] Advanced clinical studies are urgently needed to clarify the potential synergistic effects of irradiation therapy in combination with such agents in patients with metastatic gastrointestinal cancer.

SURROGATE MARKERS OF ANTIANGIOGENESIS IN GASTROINTESTINAL ONCOLOGY

With new antiangiogenic treatment strategies emerging and novel compounds being tested in advanced clinical studies, it is now apparent that novel surrogate markers reflecting the biological activity of the tested agents are important for their successful development. Several attempts have been undertaken to define the biological effects obtained by administration of antiangiogenic therapy. However, it has to be emphasised that no adequate surrogate markers of any antiangiogenic treatment strategy have been identified to date and therefore circulating marker levels do not necessarily reflect intratumoral ongoing angiogenesis.

Multiple assays to assess the effects of antiangiogenic effects of novel compounds in clinical trials have been proposed (for example, measuring levels of angiogenic factors in body fluids, including serum, plasma, and urine). It has to be kept in mind however that circulating growth factor levels in serum are not necessarily in total derived from

Table 4 Antiangiogenic small molecule compounds in gastrointestinal solid tumours: active studies

Compound/trial characteristics	Trial identifier
PTK787/ZK222584 (Vatalanib)/multiple receptor associated tyrosine kinases	
Phase II, advanced pancreatic cancer, open label	NCT00226005
Phase II, metastatic solid tumours, open label	NCT00171587
AG-013736/multiple receptor associated tyrosine kinases	
Phase II, advanced pancreatic cancer, versus gemcitabine monotherapy	NCT00219557
AZD2171/VEGFR-2 associated tyrosine kinases	
Phase I, stage IV colorectal cancer, versus conv. chemotherapy	NCT00107250
Phase II, colorectal cancer, versus conv. chemotherapy + bevacizumab	NCT00278889
BAY 43-9006 (Sorafenib)/*raf* protein kinases	
Phase II, stage III and IV colorectal cancer, versus cetuximab/irinotecan	NCT00134069
XL999/multiple receptor associated tyrosine kinases	
Phase II, metastatic colorectal cancer, open label	NCT00277303

the primary tumour. For example, particulate blood elements, including platelets and neutrophils, represent important compartments for circulating VEGF.[168] Therefore, specimen handling is of crucial importance in order to reflect actual serum concentrations of the marker tested.

Further approaches include histological analysis of tumour biopsies (such as laser scanning cytometry), and biological and radiological imaging of tumour associated angiogenic activity (including three dimensional ultrasound, magnetic resonance imaging, computed tomography, and positron emission tomography using ^{15}O-H_2O and ^{18}FDG as tracer substances) also appear to represent markers for evaluation of antiangiogenic therapy.[169] Recent studies have suggested that ex vivo analysis of circulating endothelial progenitor cells (CEP) and circulating endothelial cells might be useful in determining the angiogenic activity of human tumours in treated patients. Evidence for this hypothesis comes from a study showing that patients with progressive cancers display significantly higher levels of CEPs compared with healthy controls.[170] In humans, levels of CEPs appear to be dependent on expression of VEGF.[171] Several attempts have been made to identify surrogate markers in clinical trials using antiangiogenic agents in treated colorectal cancer patients, including monitoring of microarray based gene expression profiles of patient peripheral blood mononuclear cells.[172] A multitude of serum markers reflecting tumour associated angiogenesis in colorectal cancer patients with extensive and metastatic disease have been reported. In colorectal cancer patients, serum MMP-2 and -9 levels were associated with metastasis and tumour invasion and were therefore proposed as potential surrogate markers in antiangiogenic therapy.[173] As for VEGF, serum levels of bFGF were reported to be elevated in colorectal cancer patients with extensive or metastatic disease.[174]

Assessment of circulating VEGF levels in colorectal cancer patients

Several studies have reported on the assessment of serum VEGF levels in colorectal cancer patients, showing ambiguous results regarding the correlation of peripheral cytokine levels with clinical angiogenic activity.[175] Although correlations were observed for tumour size and volume, with higher circulating VEGF levels, its exclusive use as a diagnostic tumour marker is limited mainly due to low sensitivity.[176] Other investigators have reported that T2–T4 tumour stages of colorectal cancer can be detected by elevated VEGF serum levels.[177 178] Similarly, serum VEGF levels were reported to be associated with tumour stage, the presence of lymphogenic and distant metastasis, and depth of tumour invasion.[179 180] In these patients, serum VEGF levels were shown to decrease after curative, but not palliative, resection.[179] In patients having undergone curative surgery for colorectal cancer, the combination of high VEGF and high carcinoembryonic antigen (CEA) levels six months after resection was strongly associated with a poor prognosis and disease recurrence.[181] On the other hand, treatment strategies employing monoclonal antibodies against VEGF receptor 2 (KDR) were shown to elevate circulating VEGF levels in treated tumour bearing mice, possibly by competitive antagonism.[169] Similarly, the use of bevacizumab in patients with metastatic renal cancer was associated with a significant increase in plasma VEGF levels.[182] Elevated VEGF levels might therefore serve as a surrogate marker for determining the optimal biological dose of antibody administration in these patients.[183] Recent studies have indicated that elevated circulating VEGF levels in colorectal cancer patients might in fact be derived from cellular compartments other than tumour cells (that is, leucocytes and activated platelets). Evidence for this hypothesis stems from studies showing that extracellular VEGF might accumulate in corpusculate fractions of peripheral blood from patients and subsequently be liberated into the supernatant depending on sample storage conditions.[184] In a recent study, Ranieri *et al* have reported that activated platelet rich plasma anticoagulated with sodium citrate/adenosine/dipyridamole (P-APRCTAD) represents the peripheral blood fraction most suitable to distinguish healthy controls from colorectal cancer patients by peripheral VEGF levels.[185] Further studies will be needed to precisely define the role of VEGF levels in monitoring disease activity and efficacy of antiangiogenic treatment.

- To date, there are no validated surrogate markers to monitor antiangiogenic therapy.

Other potential angiogenesis markers in colorectal cancer patients

Further attempts have been made to identify molecules involved in angiogenesis as surrogate markers. Elevated plasma levels of matrix metalloproteinases -2 and -9, key enzymes involved in the degradation of the basement membrane and the extracellular matrix in tumour invasion and angiogenesis, were reported to be associated with advanced tumour stage in colorectal cancer patients, both

decreasing to levels within the normal range following curative surgery.[173] Angiogenin, an angiogenic peptide initially identified in culture supernatants of a colorectal cancer cell line, was found to be elevated in the serum of colorectal cancer patients and correlated with disease stage.[186] Soluble FLT1 (sFLT), a natural antagonist of circulating VEGF, is detectable in the sera of colorectal cancer patients, but not healthy controls. Interestingly, sFLT levels did not show any significant correlation with serum VEGF levels.[187] Similarly, levels of soluble E-selectin, an endothelial cell adhesion molecule involved in angiogenesis, displayed higher serum levels in metastatic colorectal cancer patients compared with normal controls. In these patient groups, elevated levels of soluble E-selectin were not correlated with circulating serum markers of systemic inflammation, including C reactive protein, TNF-α, and fibrinogen.[188]

Other groups have suggested that molecular imaging of tumour microvasculature using dynamic contrast enhanced magnetic resonance tomography might serve as a potential non-invasive technique to monitor antiangiogenic therapy in colorectal cancer patients.[189]

Recent investigation has indicated that the process of angiogenesis is dependent on the equilibrium of fibrinolysis and fibrin polymerisation.[190 191] As a prerequisite for neovascularisation, the breakdown of ECM proteins, including cross linked fibrin, appears to be a fundamental step in the growth of tumour associated microvessels. Crosslinked fibrin contained in the ECM represents a stable framework for the migration of endothelial cells during angiogenesis and for tumour cells during invasion. D-dimers are fibrin degradation fragments which occur in the serum of patients when both intravascular and extravascular crosslinked fibrin is degraded by plasmin.[192] As a routinely assessable serum marker, D-dimers are clinically used to screen for thromboembolic events such as pulmonary embolism and deep venous thrombosis. In patients suffering from solid tumours, including lung, prostate, cervical, and colorectal carcinoma, elevated plasma D-dimer levels have been shown to be directly correlated with levels of established tumour markers such as CA 125 and CEA. Moreover, in colorectal cancer patients, serum D-dimer levels were found to correlate with depth of tumour invasion and lymph node involvement.[192–194]

Similar observations have been made by our group in the antiangiogenic treatment of benign intra-abdominal desmoid lesions using interferon beta and the selective oestrogen receptor modulating agent toremifene. In two patients suffering from extensive intra-abdominal desmoid disease, treatment with these two agents was associated with an immediate and sustained drop in circulating D-dimer levels which was accompanied by marked tumour regression and decreasing microvessel density, as assessed in post treatment desmoid tissue.[195]

CONCLUSIONS

Angiogenesis associated with gastrointestinal tumours is a multistep sequence dependent on a plethora of biological and physical factors, including cytokines, chemokines, hypoxia, and tissue pH. Even though blockade of single angiogenic factors has yielded amazing results in a wide variety of rodent preclinical models of gastrointestinal cancers, most of the substances tested have shown disappointing efficacy in the setting of clinical phase II and III studies in the treatment of human gastrointestinal cancer. This is in part due to the fact that rodent vascular biology is different to human physiology in many aspects. On the other hand, fast growing malignant human tumours possess the ability to develop resistance towards blockade of single angiogenesis pathways while utilising the next weakest link in the chain of angiogenic events. Given the multitude of possibilities which tumours use to satisfy their excessive demands of oxygen and nutrients, successful angiogenic therapy will likely consist of a combination of established cytotoxic chemotherapeutic and biological agents. Recent advances in the treatment of colorectal carcinoma using 5-FU based chemotherapy regimens in conjunction with strategies based on the blockade of circulating VEGF support this hypothesis. The use of adjuvant strategies to enhance the efficacy of modern antiangiogenic therapy will have to be assessed in clinical studies. Those will potentially include selective COX-2 inhibitors, thalidomide, and radiotherapy.

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EDITOR'S QUIZ: GI SNAPSHOT

Answer

From question on page 1495

Eosinophilic oesophagitis (EO) is an increasingly recognised condition in adults presenting with dysphagia and chest pain. Although this condition is well recognised and described in paediatric patients, it is under recognised in adults. There appears to have been a general lack of desire among adult gastroenterologists to take biopsy specimens from the ''normal'' oesophagus. The established diagnostic criterion of EO is a dense infiltration of the epithelium with eosinophils (>15/high power field). Distal oesophageal biopsy alone may miss the diagnosis of EO as eosinophil infiltration is not uniform. Therefore, biopsies should be obtained from the distal and mid-oesophagus. Several endoscopic features such as small calibre oesophagus, white vesicles or papules, proximal stenosis, and rings have been associated with EO. Tearing or even perforation of the oesophagus can occur with simple passage of the endoscope even in the absence of overt rings. Endoscopic findings can be normal or very subtle in these patients, and the findings can easily be missed during endoscopy.

Therefore, in patients undergoing evaluation for dysphagia, caution should be exercised even in the macroscopically normal oesophagus. Careful inspection is required both on insertion and withdrawal of the endoscope.

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