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Review

Angiogenesis of gastrointestinal tumours and their metastases – a target for intervention?

G. Garcea [a,*], T.D. Lloyd [b], A. Gescher [a], A.R. Dennison [b], W.P. Steward [a], D.P. Berry [b]

[a] *Cancer Studies and Molecular Medicine, 5th Floor, The Robert Kilpatrick Clinical Sciences Building, The Leicester Royal Infirmary, Leicester LE2 7LX, UK*

[b] *Department of Hepatobiliary Surgery, The Leicester General Hospital, Gwendolen Road, Leicester, UK*

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Abstract

Angiogenesis is an obligatory event for the growth of tumours beyond 2 mm in diameter, above which simple oxygen diffusion can no longer support the rapid proliferation of malignant cells. Angiogenesis is a fine balance between inhibitory and stimulatory factors, the knowledge of which offers novel targets for the treatment of gastrointestinal neoplasia. A literature search of Pubmed and Medline databases was undertaken, using the keywords colorectal cancer, pancreatic cancer, gastrointestinal cancer, angiogenesis and anti-angiogenesis therapy. It was found that angiogenesis in primary tumours is a sequential and highly complex cascade of molecular events resulting in the rapid exponential growth of the tumour. Hepatic metastases of primary tumours may be less reliant on traditional angiogenic pathways, by co-opting pre-existing hepatic vasculature. Research into angiogenesis has revealed many different sites that can be targeted by agents such as tyrosine kinase inhibitors. Many anti-angiogenic agents are undergoing preclinical evaluation, with only a few entering phase I and phase III clinical trials. However, early results suggest that anti-angiogenic therapy could be an important adjunct to conventional chemotherapy treatment of gastrointestinal neoplasia.

Keywords: Colorectal cancer; Pancreatic cancer; Gastrointestinal cancer; Angiogenesis and anti-angiogenesis therapy

1. Introduction

Angiogenesis refers to the process by which a new blood supply is established from pre-existing blood vessels [1]. The proliferative index of a tumour decreases with increasing distance from the nearest capillary blood vessel, and the rapid exponential growth of tumours is dependent on vascularisation of the tumour mass [2–4]. Without angiogenesis, tumours are limited in size to the distance that oxygen can diffuse, namely 1–2 mm. Increased vascularity not only allows an expansion in tumour size, it also leads to a greater probability of haematogenous embolisation of the tumour and so metastatic spread. Tumour foci in distant organs are also reliant on angiogenesis to establish an independent blood supply. Hence, disruption of the angiogenesis pathway offers novel opportunities in the management of solid gastrointestinal-tract tumours.

2. Angiogenesis in primary gastrointestinal tumours

Angiogenesis is initiated by pro-angiogenic stimuli, which include acidic pH, cytokines, growth factors, activation of oncogenes or hypoxia. Angiogenesis is dependent on the balance between stimulatory and inhibitory factors. Pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), bind to sites on endothelial cell membranes that lead to the proliferation of endothelial cells and subsequent invasion of the basement membrane. Proliferating endothelial cells eventually form capillary tubes, which connect and form a vascular network (Fig. 1). Blood vessels in tumour tissue are thin walled, highly disorganised, with fewer supporting cells such as pericytes and smooth muscle cells, than normal tissue vasculature [5,6]. Tumour angiogenesis depends on

* Corresponding author.
E-mail address: gg43@le.ac.uk (G. Garcea).

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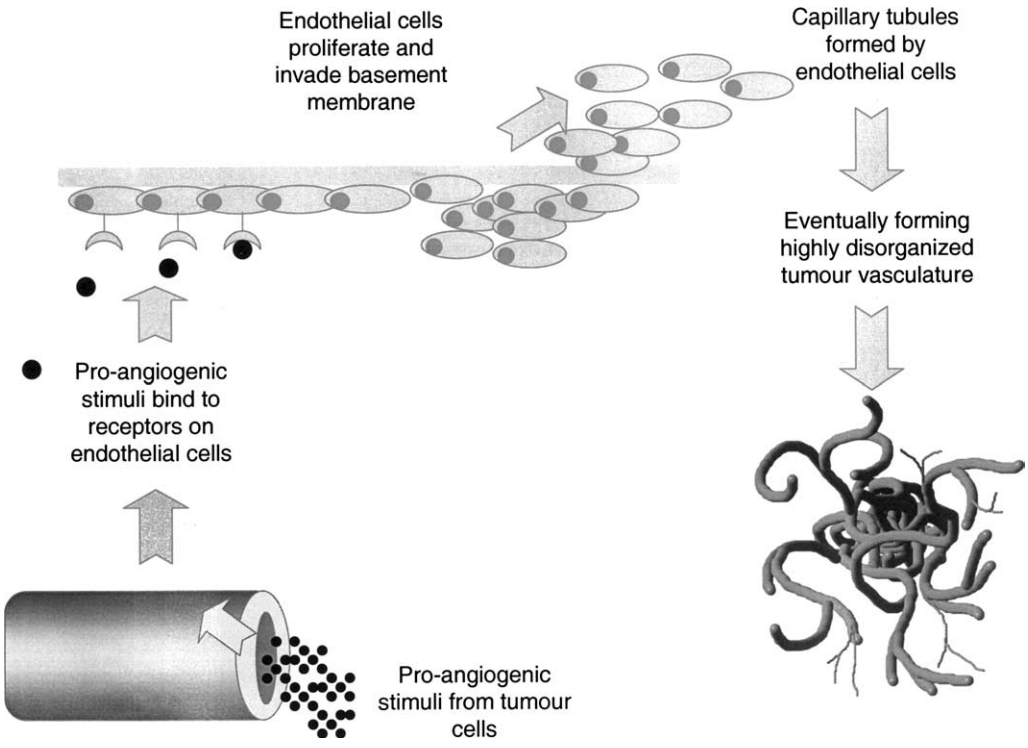

Fig. 1. Sequence of events describing the angiogenic process.

the expression of specific factors that initiate the cascade of events leading to microvasculature formation. Numerous growth factors that promote angiogenesis have been identified; selected factors germane to gastrointestinal carcinogenesis are detailed below.

2.1. Vascular endothelial growth factor

The VEGF family comprises six molecules classified as VEGF-A, -B, -C, -D, -E and placenta growth factor (PIGF) [7]. Receptors for VEGF-A are expressed almost exclusively on endothelial cells and possess tyrosine kinase activity. Five isoforms of VEGF-A have been described [8] (Fig. 2). VEGF-A stimulates endothelial cell proliferation and acts as an antiapoptotic agent [9,10]. VEGF-A also possesses vasodilatory abilities [11] and stimulates the synthesis of proteolyic enzymes [12]. VEGF-A is 50000 times more effective than histamine at inducing vascular permeability, which allows diffusion of proteins into the interstitium, to form a lattice network onto which endothelial cells migrate [13]. Three VEGF-A receptors have been identified; VEGFR-1, VEGFR-2 and VEGFR-3 [14]. Each receptor may function to mediate distinct intracellular functions: for example, VEGFR-1 functions predominantly in the migration of endothelial cells and VEGFR-2 induces permeability and proliferation of endothelial cells [13].

VEGF-A expression is upregulated in colorectal adenocarcinomas [15–17]. A high VEGF-A expression is associated with an increased likelihood of metastatic spread of primary colorectal tumours [16,18] and is inversely associated with long-term patient survival [16,19,20]. In gastric malignancies, VEGF-A expression is reportedly correlated with microvessel density [21], lymphatic and haematogenous invasion [22,23], and poor prognosis [24]. VEGF-A expression in pancreatic adenocarcinoma is associated with the likelihood of disease progression, poor prognosis and increased risk of metastatic spread [25–29].

2.2. Platelet-derived growth factors

Platelet-derived growth factors (PDGF) act as chemoattractants and stimulate the motility of mesenchymal cells such as fibroblasts and smooth muscle cells [30]. Five isoforms of the PDGF family exist, which include PDGF-AA, PDGF-AB, PDGF-BB, PDGF-C [31] and PDGF-D [32–35]. PDGFs comprise of homo- and heterodimers of disulphide-bonded A- and B-polypeptide chains [30]. PDGF-BB is similar in its three-dimensional structure to VEGF, nerve growth factor, and transforming growth factor-β (TGF-β) [30]. Receptors, with tyrosine kinase activity, for PDGF have two intracellular subunits labelled as PDGFR-α and PDFR-β [36]. PDGF-BB is able to bind to and activate both PDGFR-α and PDGF-β receptors, whereas PDGF-AA can only bind to PDGFR-α subunits [35] (Fig. 3). PDGF-BB is a potent oncogene with a wide

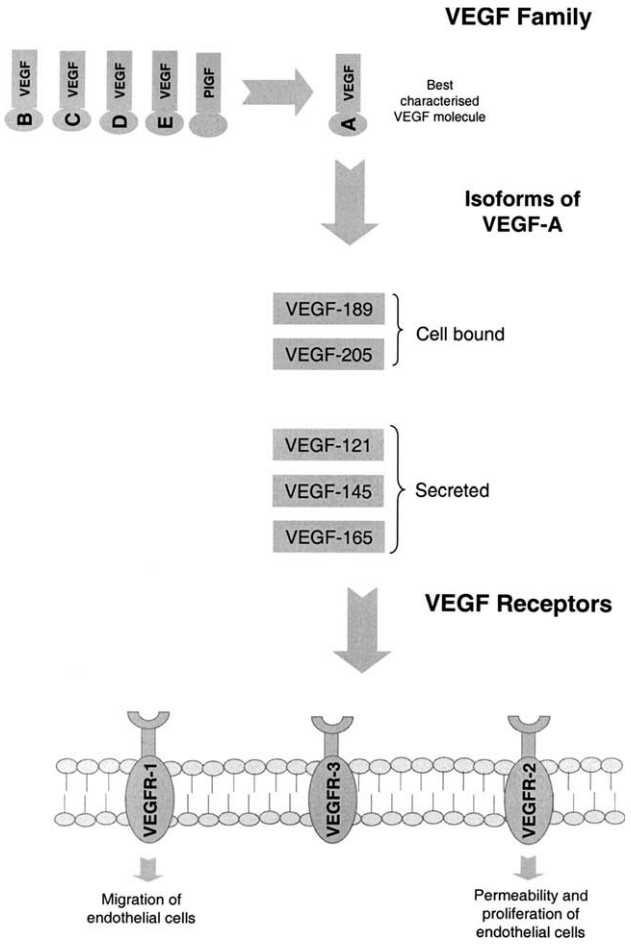

Fig. 2. VEGF family comprising of six molecules VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PIGF. VEGF-A exists as at least five different isoforms, VEGF-189 and VEGF-205 are cell bound proteins whilst VEGF-165 is the most common found in tumours. Three receptors for VEGF-A have been characterised VEGFR-1, VEGFR-2 and VEGFR-3, each of which may mediate different events in the angiogenesis pathway.

spectrum of activity including the induction of VEGF-A production, the migration of smooth muscle cells and the regulation of vasoconstriction [37,38]. PDGF-DD preferentially signals though the PDGFR-β receptor, and, although it is overexpressed in many tumour cell lines, its exact role in angiogenesis is unclear [36]. PDGF-CC mediates its effects via PDGFR-α and is over-expressed in several tumour models, including fibrosarcomas and melanomas, in which PDF-CC is associated with accelerated tumour growth [32].

2.3. Platelet-derived endothelial cell growth factor

Platelet-derived endothelial cell growth factor (PD-ECGF), thymidine phosphorylase, acts as a chemoattractant for endothelial cells [39], induces vascularisation in rat sponge models and accelerates the growth of breast cancer xenografts in mice [40]. PD-ECGF correlates with increased microvessel density in colorectal tumours and may be an important pro-angiogenic factor in individuals with a low expression of VEGF-A [41]. In gastric cancers, PD-ECGF has been related to microvessel density, proliferative index [22,23,42] and poor prognosis [23]. Furthermore, PD-ECGF and VEGF-A may be synergistic in inducing angiogenesis in certain types of gastric cancer [21]. Expression of PD-ECGF in pancreatic cancers had a negative association with long-term survival [29,43].

2.4. Angiopoietins

Four angiopoietins have been described, Ang-1 to -4, all of which act on the Tie-2 tyrosine kinase receptor found specifically on endothelial cells [44–46]. Ang-1 and Ang-4 activate the Tie-2 receptor; Ang-2 and Ang-3 block Ang-1-induced phosphorylation [45]. Biologically, Ang-1 mediates endothelial cell stability, whilst Ang-2 is involved in vascular remodelling. Ang-2 is thought to be an initiating factor in angiogenesis by priming endothelial cells for mitogenic signals [46]. In this respect, Ang-2 requires the presence of growth factors such as VEGF-A for angiogenesis to occur; without VEGF-A, Ang-2 results in vessel regression [47].

The balance between Ang-1 and Ang-2 could have a key role in tumour angiogenesis. Ang-1 expression is uncommon in colorectal tumours, whereas Ang-2 is over-expressed [48,49]. Ang-2-transfected colon cancer cells implanted into immunocompromised mice exhibited an accelerated rate of growth when compared to control cells [50]. Ang-2 expression in human gastric cancers has been negatively correlated with survival [51]. In animal models of gastric carcinogenesis, Ang-2 transfection resulted in tumours that were highly likely to metastasise [51]. In patients with pancreatic tumours, serum Ang-2 was increased when compared to normal volunteers, and patients with the highest concentrations of serum Ang-2 had reduced survival [52].

2.5. Fibroblast growth factors

Fibroblast growth factors (FGFs) comprise a family of 20 molecules with a wide range of biological effects; among their actions is the ability strongly to stimulate angiogenesis [53]. The two most extensively studied FGFs in relation to angiogenesis are FGF-1 (also known as acidic fibroblast growth factor; aFGF) and FGF-2 (basic fibroblast growth factor; bFGF) [54]. FGFs mediate their effects via four tyrosine kinase receptors, FGFR-1 to -4 [55] (Fig. 4). FGFR-1 is the main receptor expressed in endothelial cells in vitro, but small amounts of FGFR-2 have also been found in endothelial cells [56]. Activation of FGFRs induces endothelial cell proliferation, migration and tubulogenesis [57–60]. Many tumour cell lines synthesise FGF-1 or FGF-2;

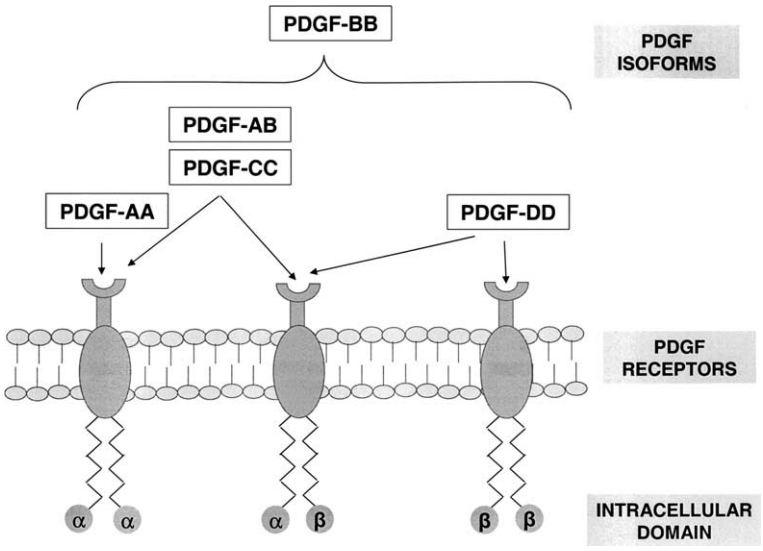

Fig. 3. Binding affinity of the different PDGF isoforms for the different PDGF receptors. PDGF-BB can bind to both α and β homodimers, PDGF-CC can only bind to PDGF-Rα homodimers, although there is some evidence that it can activate PDGFRαβ heterodimers. Binding characteristics for PDGF-AA, PDGF-AB and PDGF-DD.

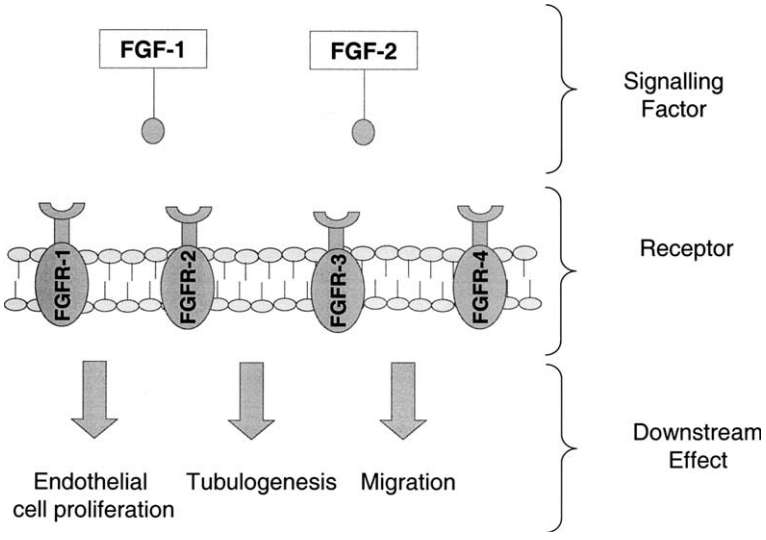

Fig. 4. Outline of FGFs and FGF receptors and down-stream signalling effects.

FGF-2 concentrations are raised in the urine of patients with a wide spectrum of different cancers [61,62].

FGFs were over-expressed in mesenteric blood samples from patients with colorectal tumours [63]. In colon cancer cell lines, the addition of FGFs increased proliferation and invasiveness when compared to controls [64]. In human subjects with colorectal tumours, elevated FGF-2 was associated with increased likelihood of metastatic spread of colorectal tumours [65] and a positive association has been reported between the expression of FGFs and stage D colorectal tumours [66]. FGFs have been linked to the resistance of tumours to chemotherapy [67]. Furthermore, serum FGF-2 (bFGF) may have a predictive value for disease progression in patients with untreated metastatic colorectal cancer [68].

FGF is over-expressed in surgical specimens from gastric carcinomas [69]. A positive association was reported between patients with the highest expression of FGF and tumour invasiveness [70], and lymph-node metastases [71]. FGF-2 (bFGF) expression in gastric carcinomas may also predict recurrence following resection [72,73]. FGFs play an important part in pancreatic tumour angiogenesis [74–76] and it has been suggested that measuring FGF could be utilised clinically to predict patient survival and the likelihood of metastatic spread [77].

2.6. Hypoxia-inducible factor

Hypoxia-inducible factor-1 (HIF-1) plays a critical part in oxygen homeostasis. The HIF-1 protein consists of two subunits, HIF-1α and HIF-1β [78]. HIF-1α is normally degraded rapidly by proteasomes under normotensive conditions. However, during hypoxia, HIF-1α destruction is suppressed and the accumulation of HIF-1α leads to the transactivation of genes whose protein products act to increase tissue oxygen. The genes activated include those coding for erythropoietin, glycolytic-pathway enzymes, haem oxygenase, carbonic anhydrase and the pro-angiogenic growth factor VEGF [79–82]. Thus, HIF-1α serves as a pro-angiogenic factor acting upstream from VEGF. HIF-1α protein has been found in a number of cancer cells lines, including colorectal cancer and has been associated with tumour vascularisation in vitro [83–85] and with increased likelihood of invasion [86]. In human colorectal cancer tissue, HIF-1α protein correlated with tumour staging [87] and with VEGF expression [87,88]. In pancreatic cancers, HIF-1α protein regulated VEGF expression [89] and rendered pancreatic cells resistant to hypoxia-induced apoptosis, possibly via the activation of anaerobic metabolism [90].

2.7. Interleukin 8

Interleukin 8 (IL-8) expression is regulated by hypoxia and acidosis in the tumour microenvironment. IL-8 can directly stimulate tumour growth [91,92], but may also stimulate tumour angiogenesis [93]. IL-8 concentrations in tumour tissue and serum of patients with colorectal tumours are reportedly correlated with microvessel density and significantly higher in patients with hepatic metastases of their primary tumours, when compared to patients without metastases [94]. In gastric carcinoma cell lines, transfection with IL-8 resulted in rapidly growing, highly vascular tumours when compared to controls [95,96]. In clinical studies of patients with gastric tumours, those with the lowest survival rates had the highest expression of IL-8 in their tumour specimens [97]. Data for pancreatic tumours are limited, but one study has reported enhanced invasiveness of pancreatic tumour cell lines following treatment with exogenous IL-8 [98].

2.8. Integrins and extracellular matrix

The integrins are a family of cell-adhesion receptors and comprise a heterodimer complex of two transmembrane subunits, α and β. To date, 18-subunits and eight β-subunits are described, giving rise to 24 heterodimers [99,100]. Integrins provide the physical interaction with the extracellular matrix necessary for endothelial cell adhesion, survival, proliferation and differentiation [101]. In human gastric cancers, over-expression of integrins is associated with metastatic spread and is also an independent predictor of patient survival [102]. Furthermore, agents that inhibit integrin expression and function result in improved survival and disease dissemination in animal models of metastases [102,103].

2.9. Thrombospondin

Thrombospondin (TSP) inhibits angiogenesis and is released by platelets in the presence of thrombin [104]. It is also produced by fibroblasts, vascular smooth muscle cells, monocytes and macrophages.

Five subtypes of TSP have been characterised thus far, of which TSP-1 and -2 have been studied most extensively in relation to the inhibition of angiogenesis. In colorectal tumours TSP-1 is mostly localised to fibroblasts [105] and was inversely correlated with tumour vascularity and likelihood of recurrence [106]. TSP-2 inhibited vascular proliferation in colorectal cancer cell lines [107] and had an inverse correlation with prognosis and survival in patients with colorectal cancer [108]. In both gastric and pancreatic cancer, expression of TSP-1 correlates with microvessel density [109,110], and in the case of pancreatic cancer TSP-1 has been shown to be a good prognostic indicator [111].

3. Resistance to hypoxia

Numerous other growth factors exist that stimulate or inhibit angiogenesis. Angiogenesis is driven by the tumour's response to hypoxia, and there is growing evidence that the resistance to hypoxia conferred by enzymes such as haem oxygenase and carbonic anhydrase also plays an important part in the proliferation of solid tumours, as briefly discussed next.

3.1. Haem oxygenase

Haem oxygenase (HO) is the rate-limiting enzyme in haem degradation resulting in the production of biliverdin, which is subsequently reduced to bilirubin by biliverdin reductase [112]. Two forms of HO have been described; HO-2, which is constitutively expressed, HO-1, which is inducible in response to a variety of different stimuli, such as ultraviolet light, heat shock, nitric oxide or hypoxia [60,113–115]. HO-1 induction offers cellular protection against hypoxia because the biliverdin produced has free radical-binding activity. Hence, over-expression of HO-1 in solid tumours may confer a survival advantage by preventing apoptosis [116] and conferring resistance to tumour cells against hypoxic stress [117].

3.2. Haem oxygenase

The Haem oxygenases (HOs) are group of transmembrane enzymes that catalyse the reversible hydration of carbon dioxide to carbonic acid [118]. Twelve isoenzymes have been characterised thus far, CA I to XI and more recently CA XII. The exact role of CAs in solid tumour growth is still unclear, but they could act as protectants against hypoxic damage. CA I, II, IX and XII are correlated with the biological aggressiveness in colonic cancers [119–122]. Attempts have also been made to use faecal concentrations of CA II as a screening tool for colorectal cancer [123].

4. Angiogenesis in liver metastases

The liver is the most common site of metastases from gastrointestinal tumours. It receives 30% of the cardiac output and all venous drainage from the gastrointestinal tract passes through it. Tumour cells from gastrointestinal sources are therefore likely to embolise to the dense capillary network within the liver parenchyma. The microenvironment of the liver also favours angiogenesis by providing a rich matrix of endothelial cells, inflammatory cells, Kuppfer cells and fibroblasts. Metastasis of primary tumours to the liver is a sequential progress leading to a population of metastatic cells within the liver parenchyma [93] (Fig. 5).

Two types of vasculature have been described in liver metastases, a 'sinusoidal pattern', with convoluted vessels lacking a basement membrane, in which metastatic cells are located between the hepatocytes and sinusoidal endothelial cells, and a 'portal type' with a high microvessel density and a basement membrane [124]. In the sinusoidal type the tumour microvessels are continuous with the sinusoidal system and contain extensive central necrotic areas. In the portal type, metastases are located near the portal tracts, with no necrotic areas, and it has been suggested that their main blood supply originates from the hepatic artery [124]. The formation of vascular supply in liver metastases is likely to differ in some important ways from angiogenesis in the primary tumours. The liver is a highly vascular organ, and it is possible that hypoxia is less important in the development of new blood vessels than in traditional angiogenesis models [125]. There is also evidence that VEGF expression is lower in hepatic metastases when compared to both the primary cancers [126] and extrahepatic abdominal metastases [127].

Certain liver metastases display a 'replacement pattern' [128], which is characterised by minimal angiogenesis and co-option of existing liver vasculature, and may share characteristics with the sinusoidal type of metastatic spread described by Paku and Lapis [124]. The other two types of pattern reported are 'desmoplastic' and 'pushing growth'. The desmoplastic growth pattern has similar characteristics to the portal type of metastatic growth described observed by Paku and Lapis. Both desmoplastic and pushing growth patterns'' had higher microvessel density counts and more immature blood vessels than in the replacement pattern. In patients with more than one liver metastasis, all of the lesions displayed the same growth pattern. The difference in growth patterns could be explained by differences in the growth patterns of the original primary

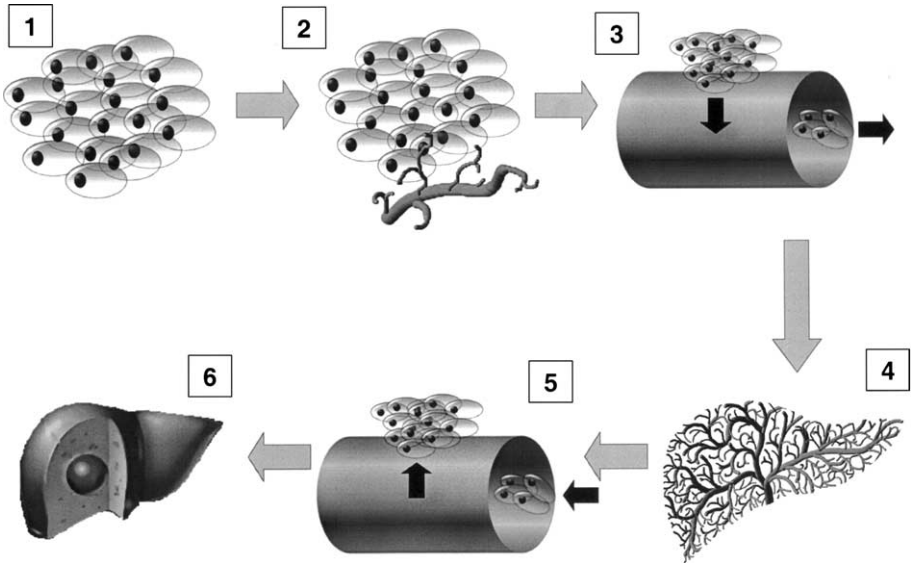

Fig. 5. Events in metastatic dissemination of gastrointestinal tumours: (1) Growth of primary solid tumours. (2) Release of pro-angiogenic fcators. (3) Malignant cells invade vascular vessels and embolise into the peripheral circulation. (4) Malignant cells lodge in capillary bed of liver. (5) Adherence, extravasation of malignant cells into liver parenchyma. (6) Establishment of population of metastatic tumour cells.

tumours, but it is possible that liver metastases are a heterogeneous group with different growth patterns and different reliance on angiogenesis. This observation is particularly relevant for the development of anti-angiogenesis agents.

5. Anti-angiogenesis therapy

When the number of capillaries per volume of tissue decreases, tumour cell proliferation is not affected but the apoptotic fraction increases, leading to 'dormant' tumours [129]. Hence, because anti-angiogenesis therapy can only inhibit tumour growth, it is likely that it will be combined with traditional chemotherapy and/or surgery. Potential sites of action for anti-angiogenesis agents are shown in Fig. 6.

5.1. Tyrosine kinase inhibitors

A staggeringly large number of different agents are currently under assessment in preclinical models and phased clinical trials. The tyrosine kinase inhibitor SU5146, 3-[2-(4-dimethylpyrrol-5-yl)methylidenyl]-indolin-2-one, is a small molecule designed to inhibit VEGFR-2 tyrosine kinase activity. SU5146 caused a marked growth inhibition in a number of experimental tumours [130,131] and phase III clinical trials are currently further evaluating the drug in patients with liver metastases from colorectal cancer [132]. SU6668, (*Z*)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid is another tyrosine kinase inhibitor that exerts inhibitory activity across a wide range of receptors including VEGF, FGF and PDGF [133]. SU6668 has undergone preclinical assessment in animal models of pancreatic and colorectal cancer, and has been shown to be a potent inhibitor of angiogenesis [131,133,134].

Other tyrosine kinase inhibitors include PTK 787/ZK222584, 1-[4-chloroanilino]-4[pyridylmethyl]phtalazinedihydrochloirde and PKI 166, 4-(*R*)-phenethylamino-6-(hydroxyl)phenyl-7H-pyrrolo[2.3-d]-pyrimidine. PKI 166 has shown promise in animal models of pancreatic cancer by increasing apoptosis and decreasing microvessel density in tumours. This effect was potentiated by using a combination of PKI 166 and gemcitabine [135,136]. PTK 787/ZK222584 has progressed through preclinical evaluation in gastrointestinal tumour models and is currently undergoing phase I clinical trials; preliminary data suggest it is well tolerated, with surrogate endpoint evidence of a biological response [137].

5.2. Antibody therapy

Antibodies targeted against the VEGF receptor have been evaluated in animal models of primary colorectal cancer; a reduction in tumour vascularity and increased endothelial cell apoptosis were demonstrated [138–140]. VEGFR antibodies were also efficacious in pancreatic tumour models [141] and murine gastric cancer models [142]. VEGF antibodies are currently undergoing preclinical assessment for the inhibition of metastasis of colonic cancers [143]; in gastric cancers transplanted to

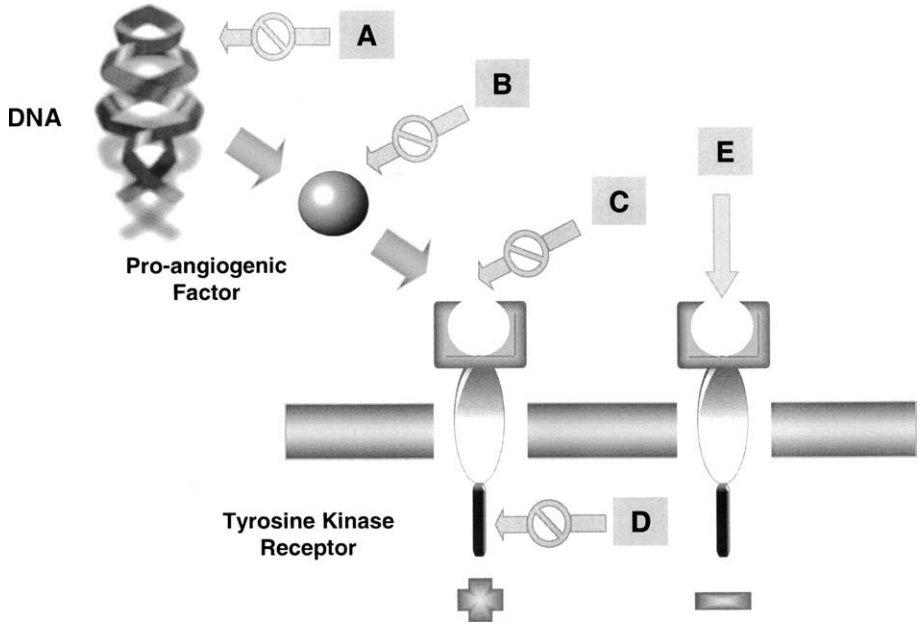

Fig. 6. Target of action of anti-angiogenesis agents: (A) Anti-sense oligonucleotides, blocking transcription of pro-angiogenic factors. (B) Antibodies specific against pro-angiogenic factors. (C) Antibodies directed against receptors. (D) Tyrosine kinase blockers. (E) Exogenous inhibitors of angigenesis.

nude mice [144], a reduction in growth, associated with reduced microvessel density, was observed.

5.3. Other anti-angiogenesis targets

Angiostatin is a kringle-containing fragment of plasminogen and is a potent inhibitor of angiogenesis. It inhibited the growth of hepatic metastases of pancreatic origin in nude mice [145] and of colorectal origin [146]. Transfection of gastric cancer cell lines with angiostatin cDNA, prior to transplantation into nude mice, inhibited tumorigenesis [147]. Thrombospondin has undergone early in vitro assessment using colorectal cancer cell lines [148]. Chemically stabilized ribozymes are nuclease-resistant RNA based oligonucleotides that selective bind and cleave target RNAs. Ribozymes that target the mRNA of VEGF receptors have been found to inhibit liver metastases in animal models [149]. Antisense oligonucleotides target the DNA coding for specific angiogenesis growth factors. A possible target in the application of antisense oligonucleotides for inhibiting angiogenesis would be VEGF receptors. VEGFR antisense DNA inhibited the peritoneal dissemination of human gastric cancer in nude mice [150]. Integrin antagonists are currently under assessment in phase I and II trials [151], following encouraging results in preclinical work where integrin antagonists were found to inhibit colorectal metastatic spread to the liver [152].

6. Conclusion

Angiogenesis is essential in the growth and metastasis of gastrointestinal tumours. Research into angiogenesis has afforded clinicians multiple new targets for anticancer drugs. An important consideration in the development of such agents is the knowledge that hepatic metastases differ from primary gastrointestinal tumours in their growth patterns and dependence on angiogenesis. Anti-angiogenic therapy has the potential to become an important adjunct to present treatment modalities for solid gastrointestinal tumours, but until the results of phase III trials become known, the application of anti-angiogenic agents in clinical practice is yet to be fully realised.

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