

Angiogenesis as a therapeutic target in cancer

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New blood vessel formation (angiogenesis) is a physiological process which is active in embryogenesis, in adults during wound healing and in the female reproductive cycle. Physiologically, it is a tightly regulated and transient process under the control of a host of pro- and anti-angiogenic factors. The process of angiogenesis is critical in cancer for growth and metastasis. Tumours cannot grow beyond 200–300 μm in diameter unless they recruit new blood vessels to supply nutrients and oxygen. This dependence on new vessel formation and the relative genomic stability of 'normal' endothelial cells (ECs) make angiogenesis an ideal target for the treatment of cancer.¹

The tight control of new vessel formation is attained by a fine balance between various pro- and anti-angiogenic factors normally present in the body. In response to changes in their micro-environment, cancer cells trigger 'the angiogenic switch' to an 'on' position whereby pro-angiogenic markers predominate.² Hypoxia is a

key trigger, but another important factor is the changes in cell-to-cell interactions that take place in the highly proliferative state that exists in tumours which is augmented by the inflammatory and immune responses to cancer tissue. Specific clones of cancer cells that harbour oncogenic mutations that stimulate the production of pro-angiogenic factors or deletion of tumour suppressor genes that suppress angiogenesis are positively selected.

Angiogenic mechanisms

Hypoxia occurs as tumour cells outgrow the available blood supply. Reduced oxygen levels in tumour cells and surrounding stromal cells lead to the stable production of hypoxia-inducible factor-1 which controls the expression of several genes important in cellular adaptation to hypoxia including vascular endothelial growth factor-A (VEGF-A, commonly referred to as VEGF) (Fig 1).

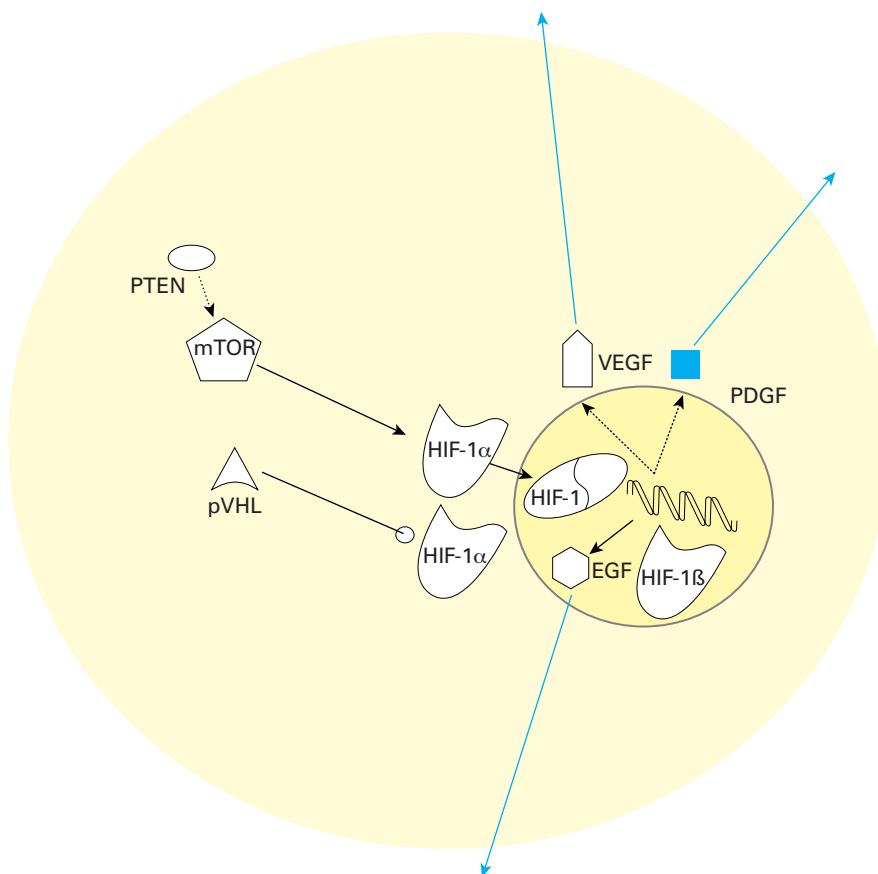


Fig 1. Hypoxia inhibits the binding of von Hippel Lindau protein (pVHL) to hypoxia-inducible factor-1 α (HIF-1 α) and prevents further degradation of HIF-1 α , thereby leading to its dimerisation and formation of HIF-1. HIF-1 leads to transcription of a number of genes involved in the hypoxia response, including the production of various growth factors like vascular endothelial growth factor (VEGF), endothelial growth factor (EGF) and platelet-derived growth factor (PDGF). Amplified signalling through phosphoinositide 3-kinase and its downstream target, mTOR (mammalian target of rapamycin) enhances HIF-1-dependent gene expression. PTEN = phosphatase and tensin homolog.

Vascular endothelial growth factor pathway

VEGF-A was initially identified as a vascular permeability factor in 1983³ and characterised as an endothelial specific mitogen by Ferrara in 1989.⁴ It is the prototypic and most important member of the VEGF family of growth factors which, together with their receptors, form one of the major pathways in tumour angiogenesis. In this family there are:

- seven individual growth factors, VEGF A-E, placental growth factor 1 and 2
- three tyrosine kinase receptors (VEGFR 1–3), to which the growth factors bind leading to receptor dimerisation and activation of downstream signalling cascades. VEGFR 1 and 2 promote angiogenesis and VEGFR 3 stimulation leads to lymphangiogenesis.

Neuropilin 1 and 2 are cell surface proteins that bind VEGF and may act as co-receptors enhancing VEGF signalling via VEGFR-1 (Fig 2).⁵

Alternative pathways involved in angiogenesis

Several other factors have also been identified as playing important roles in

angiogenesis and interacting with the VEGF pathway:

- *Fibroblast growth factor-2* (FGF-2) induces neovascularisation *in vivo* and is implicated in the growth of new blood vessels during wound healing and embryogenesis.
- *Platelet-derived growth factors* (PDGF) are expressed by activated ECs. PDGF receptors are present mainly on pericytes where the growth factors act as chemoattractants and induce mitogenesis.⁶ Pericytes play an important role in the organisation of capillaries. PDGF is important in recruiting pericytes and tumour fibroblasts, and in paracrine stimulation of stroma.
- *Angiopoietin-1* and *transforming growth factor α* impact on PDGF function and regulate the integrity of the endothelium, returning the activated ECs to a more stable state with less metabolic activity.
- The *Delta/Jagged–Notch system* is an important pathway in fetal vascular development. One of its ligands, Delta-like ligand 4 (DLL4), has been the subject of many recent studies. DLL4 is strongly expressed in tumour ECs, with much lower expression in normal ECs and tumour cells themselves.⁷

Characteristics of tumour vasculature

New blood vessels are predominantly formed by sprouting from the established vasculature or intussusception when the peri-ECs divide an existing vessel into two. Specific characteristics separate tumour blood vessels, the growth of which has been promoted by the unco-ordinated secretion of several pro-angiogenic growth factors from the normal mature vasculature. Tumour vessels tend to be tortuous, chaotic with poor lumen formation, and have structurally immature leaky walls which have reduced coverage by pericytes. The ECs of these newly formed vessels tend to be more dependent on VEGF for survival. Also contributing to angiogenesis, but their exact role has not been established, are circulating endothelial progenitor cells, highly proliferative cells derived from bone marrow, and circulating ECs, mature ECs in circulation.⁸

Clinical development of anti-angiogenic strategies in oncology

The inhibition of angiogenesis was initially proposed as a potential strategy to combat cancer in the 1970s. It has several advantages over chemotherapy and radiotherapy with which resistance due to gene mutation is a major problem. ECs that make up the tumour vasculature are essentially ‘normal’ cells with a stable genome which reduces the chances of developing resistance to treatment using classical resistance mechanisms. Anti-angiogenic agents have different toxicity profiles than cytotoxics as they specifically target the growth factors or the proliferating ECs without affecting other organs like bone marrow. The three main strategies targeting angiogenesis so far explored are:

- blocking the pro-angiogenic growth factors and specific pathways such as the VEGF pathway (antibodies to ligand, receptor, tyrosine kinase inhibitors etc)
- increasing the levels of endogenous anti-angiogenic molecules (eg endostatin)

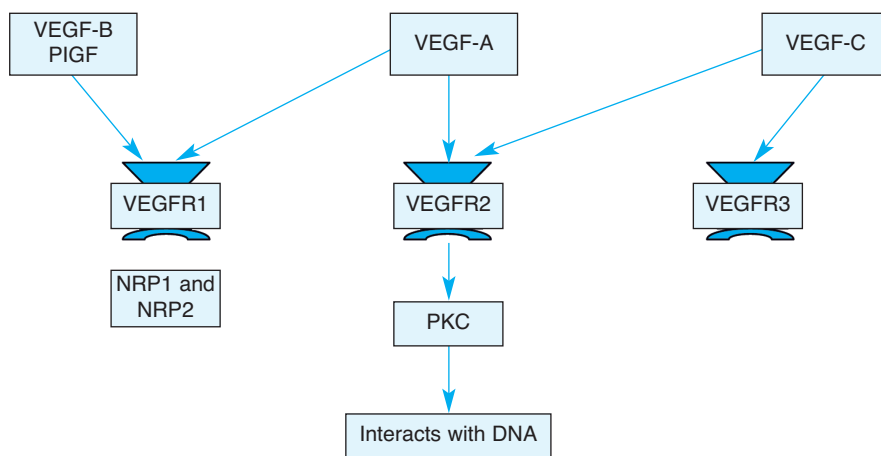


Fig 2. Vascular endothelial growth factor receptors (VEGFR) have an extracellular ligand binding domain to which the growth factors attach with an intracellular tyrosine kinase part which activates protein kinase C (PKC) downstream, thereby interacting with DNA in the nucleus. Neuropilin (NRP) 1 and 2 act as co-receptors to VEGF tyrosine kinase receptors. PIGF = placental growth factor.

- targeting the abnormalities or distinct features of the tumour vessels to disrupt vascular function (vascular disruptive agents, metronomic chemotherapy).

Targeting the vascular endothelial growth factor pathway for the treatment of cancer

The VEGF pathway was the first to be targeted through an antibody (bevacizumab) which binds the ligand, VEGF. Bevacizumab, given in combination with chemotherapy, was the first anti-angiogenic drug to be approved for use in metastatic colorectal cancer as it was associated with a survival advantage in a Phase 3 trial.⁹ Subsequent clinical trials in recurrent colorectal cancer, breast cancer, non-small cell lung cancer, liver and renal cancer have shown that the addition of bevacizumab to conventional therapy is associated with improved progression-free or overall survival.

Following the initial licensing of bevacizumab, low molecular weight VEGFR tyrosine kinase inhibitors sunitinib and sorafenib have been approved for the treatment of metastatic renal cell carcinoma.^{10,11} Sorafenib has also shown clinically significant antitumour activity in hepatocellular carcinoma.¹² The hypothesis is that VEGF inhibitors 'normalise' the tumour vasculature, and in combination with chemotherapy will lead both to more efficient delivery of the chemotherapeutic agent to the tumour cells and to sensitisation due to better oxygenation (Table 1).¹³

Table 1. Therapeutic strategies targeting angiogenesis/vascular endothelium.

Mechanisms of action	Example	Clinical use
Antibody to VEGF	Bevacizumab	Approved for use in a number of cancers
Tyrosine kinase inhibitors	Sorafenib Sunitinib	Both approved for clinical use in renal cancer, sorafenib in HCC
Soluble decoy VEGF receptor	VEGF trap	Currently in clinical trials
Vascular disruptive agents	Combretastatin ZD 6126	Currently in clinical trials
Endogenous inhibitors	Endostatin	Currently in clinical trials

HCC = hepatocellular carcinoma; VEGF = vascular endothelial growth factor.

Key Points

Angiogenesis is essential for tumour growth and metastasis

Angiogenesis is a complex process involving multiple growth factors and pathways

The vascular endothelial growth factor pathway is one of the major pathways involved in angiogenesis

Anti-angiogenic treatments have shown clinically significant efficacy in trials

Robust predictive biomarkers need to be developed and validated to identify a target population likely to respond to anti-angiogenic treatments

KEY WORDS: angiogenesis, anti-angiogenic therapy, cancer, tyrosine kinase receptors, vascular endothelial growth factor

Anti-angiogenic agents

The initial idea behind the development of anti-angiogenic agents was that resistance would not occur because of the genetic stability of ECs. This has not been borne out by the clinical trials which have largely shown that VEGF inhibitors improve progression-free and/or overall survival but do not improve cure rates. This is mainly because of the large number of growth factors involved in the complex process of angiogenesis. Several growth factors, for instance the FGF family, have been implicated in bypassing VEGF inhibition.^{14,15} Inhibitors of members of this family are currently in development.

Additional targets have also been identified which are now starting to show clinical benefit. Several drugs have been developed that target the α_v integrins; these show some single agent activity, of particular interest in glioma.^{16,17}

In addition, within the last year clinical activity has started to be seen with drugs that target the angiopoietins and hepatocyte growth factor,^{18,19} highlighting the importance of angiogenesis as a cancer target.

The most significant problem in the development of these agents is the identification of the patients who will benefit most from them. It is therefore critical that imaging and blood biomarker studies accompany the development of new anti-angiogenic agents. This issue is likely to be compounded further by the recent identification of important new targets preclinically. *In vivo* studies in which the Delta/Jagged-Notch pathway was inhibited were associated with a paradoxical increase in tumour vascularity but decrease in tumour growth. This observation was associated with a lack of vessel maturation and chaotic sprouting, resulting in poor perfusion²⁰ in contrast to the vessel 'normalisation' observed with VEGF inhibition.

Vascular disruptive agents

Proliferating ECs can also be directly targeted using vascular disruptive agents (VDA). VDAs (eg combretastatin A4) target the established vascular ECs in the tumour vasculature, leading to disruption of vascular function, tumour ischaemia and secondary tumour cell death.²¹

Metronomic chemotherapy

Another way of targeting tumour vasculature is metronomic chemotherapy in

which low, relatively non-toxic doses of chemotherapy are administered daily to target proliferating ECs, leading to their apoptosis but no significant direct tumour cytotoxicity.²²

Conclusions

The last decade has seen major advances in the field of angiogenesis, with a number of agents targeting the VEGF signalling pathway approved for clinical use after Phase 3 clinical trial evaluation. However, the optimal strategy for using anti-angiogenic agents in the clinic is still unknown. For instance, should anti-angiogenics be administered in combination with chemotherapy or as single agents for maintenance treatment? Combinations of anti-angiogenics acting on different pathways in order to prevent or delay the development of 'resistance' also need to be evaluated in preclinical and clinical trials. Most importantly, the target population likely to respond to anti-angiogenic treatment needs to be identified by developing predictive biomarkers of response/resistance using circulating biomarkers or imaging techniques such as dynamic contrast enhanced-magnetic resonance imaging.

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