Angiogenesis inhibitors in cancer – mechanisms of action

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Summary

Tumours need to develop a new blood supply to grow and metastasise. This process is called angiogenesis. Drugs that inhibit angiogenesis are therefore being evaluated in combination with chemotherapy for the treatment of various cancers. Vascular endothelial growth factor and its receptors are intimately involved in angiogenesis so they are targets for new drugs. Bevacizumab is a monoclonal antibody against vascular endothelial growth factor and is approved for use in metastatic colon cancer. Thalidomide also inhibits angiogenesis and may be used in the treatment of multiple myeloma.

Key words: bevacizumab, thalidomide, vascular endothelial growth factor.

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Introduction

Small tumours are able to grow because they can obtain nutrients and oxygen by diffusion. For tumours to enlarge further they need to develop new collateral blood vessels to provide the essential nutrients for invasion, growth and subsequent metastasis. The formation of new vessels is termed neovascularisation. This is driven by the process of angiogenesis, the sprouting of new vessels from existing vasculature.

Angiogenesis has been an appealing target for anticancer drugs for 30 years, but it is only recently that this promise has borne some fruit. There are now over 30 angiogenesis inhibitors currently in clinical trials for the treatment of malignancy (Table 1). These drugs appear to have a cytostatic rather than cytotoxic effect, leading to tumour dormancy. The available data suggest that anti-angiogenic drugs work best in conjunction with chemotherapy. Their development also involves the identification and management of a new range of toxicities.

Angiogenesis

The development of blood vessels is a complex equilibrium regulated by anti- and pro-angiogenic factors. The balance may be tilted in favour of angiogenesis by hypoxia or inflammation. This has a physiological advantage, for example in wound healing, but may be part of the pathological process in chronic inflammatory disease or cancer.

With tumour-associated angiogenesis, the cancer releases various pro-angiogenic factors (including angiogenin, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor-β (TGF-β)). These stimulate endothelial cell proliferation, migration and invasion resulting in new vascular structures sprouting from the patient's blood vessels. Cell adhesion molecules, such as integrins, are critical to the attachment and migration of endothelial cells to the extracellular matrix. The receptor for platelet derived growth factor is also important in angiogenesis as it is central to the recruitment of pericytes, the cells that surround and support capillaries.

When a tumour stimulates the growth of new vessels, it is said to have undergone an 'angiogenic switch'. The principal stimulus for this angiogenic switch appears to be oxygen deprivation, although other stimuli such as inflammation, oncogenic mutations and mechanical stress may also play a role.

The angiogenic switch leads to tumour expression of pro-angiogenic factors and increased tumour vascularisation. It is associated with more advanced tumour stages and worse prognosis in several human malignancies, including malignant melanoma and gastrointestinal, breast, prostate and lung cancers.

Vascular endothelial growth factor

Of the angiogenic factors secreted, VEGF is perhaps the most specific for endothelial cells. When VEGF binds to its receptor it triggers signalling pathways that result in endothelial cell migration, differentiation and proliferation, increased vascular permeability and release of endothelial cell precursors from the bone marrow. VEGF also prevents endothelial cell apoptosis. Higher concentrations have been associated with malignant effusions.

The VEGF-related family of genes involved in angiogenesis and lymphangiogenesis produce a number of glycoproteins, called VEGFs A–E and placental growth factors (PlGF) 1 and 2. These glycoproteins have several biologically active isoforms.

Table 1
Angiogenesis inhibitors and their targets

Drug	Target	Clinical development
Monoclonal antibody		
Bevacizumab	VEGF-A	Approved in Australia
IMC-1121B	VEGFR-2	Phase I
2C3	VEGF-A	Preclinical
Receptor tyrosine kinase inhibitors		
PTK-787	VEGFR-1, -2	Phase III
AEE788	VEGFR-2, EGFR	Preclinical
ZD6474	VEGFR-1, -2, -3, EGFR	Phase II
ZD2171	VEGFR-1, -2	Phase I
SU11248 (sunitinib)	VEGFR-1, -2, PDGFR	Phase II/III
G013736	VEGFR-1, -2	Phase II
EP-7055	VEGFR-1, -2, -3	Phase I
P-547,632	VEGFR-1, -2	Phase I/II
GW786034	VEGFR-1, -2, -3	Phase I
BAY 43-9006	VEGFR-1, -2, PDGFR	Phase III
AMG706	VEGFR-1, -2, -3	Phase I
Soluble receptor chimeric protein		
VEGF-Trap	VEGF-A, PlGF	Phase I
Inhibitors of endothelial cell proliferation		
ABT-510	Endothelial CD36	Phase I/II
Angiostatin	Various	Phase I
Thalidomide	Reduction of TNF-α	Approved in Australia
Inhibitors of integrin's pro-angiogenic activity		
Medi-522	Integrin αV	Phase I/II
EMD12194 (Cilengitide)	Integrin αV	Phase I/II
Matrix metalloproteinase inhibitors		
Marimastat	MMP-1, -2, -3, -7, -9	Phase III
Prinomastat	MMP-2, -9	Phase III
BMS 275291	MMP-1, -2, -8, -9, -13, -14	Phase III
Neovastat	MMP-2, -9, -12, VEGF	Phase III
Other		
CDP-791	VEGFR-2	Phase I
Vascular targeting drugs		
Combretastatin	Endothelin tubulin	Phase I/II
AVE8062A	Endothelin tubulin	Phase I
ZD6126	Endothelin tubulin	Phase I
AS1404	Induction of TNF-α	Phase I

Key
VEGF Vascular endothelial growth factor
VEGFR Vascular endothelial growth factor receptor
EGFR Epidermal growth factor receptor
PDGFR Platelet derived growth factor receptor
PlGF Placental growth factor
TNF Tumour necrosis factor
MMP Matrix metalloproteinases

The VEGFs are produced either by direct secretion from the tumour, or by cleavage of isoforms sequestered in the extracellular matrix by enzymes such as plasmin or the matrix metalloproteinases.

VEGFs bind to at least three receptors (VEGFR-1, VEGFR-2, VEGFR-3). The isoforms can bind with other receptors (neuropilin receptors) which may also have a role in angiogenesis. The structure of each VEGF receptor includes a kinase. For example, ms-like tyrosine kinase (Flt-1) is part of VEGFR-1. These enzymes are involved in intracellular signalling when VEGFs bind to their receptors (Fig. 1).

VEGF promotes the release of other angiogenic factors and proteolytic enzymes. The release of proteolytic enzymes results in degradation of the vascular basement membrane. New vessels are formed as endothelial cells are organised into functional tubular structures. Individual vessels then connect to form networks that allow blood to circulate. The new blood vessels formed are derived from the host and not the tumour, however they are more tortuous and leaky than normal vessels.

Angiogenesis inhibition in the treatment of cancer

To stop angiogenesis requires treatment with anti-angiogenic factors, or drugs which reduce the production of pro-angiogenic factors, prevent them binding to their receptors or block their actions. The drugs being studied can be broadly defined as those that are exclusively anti-angiogenic, such as bevacizumab, and those that have additional functions, such as thalidomide and the cyclo-oxygenase (COX)-2 inhibitors.

Endogenous anti-angiogenic factors

Endostatin is the carboxy-terminal fragment of collagen XVII. It is thought to induce apoptosis in endothelial cells and inhibition of their migration to sites of neovascularisation, probably by interfering with endothelial cell adhesion. In preclinical models, endostatin has inhibited the growth of a wide variety of human primary and metastatic tumours. Clinical trials suggest that endostatin is well tolerated, but only minor evidence of antitumour activity has been observed.

Another endogenous inhibitor of angiogenesis is angiostatin. Like endostatin, it directly induces apoptosis of endothelial cells by disrupting the normal adhesion contacts between the endothelial cells. Angiostatin also acts by inhibiting VEGF and basic fibroblast growth factor (bFGF).

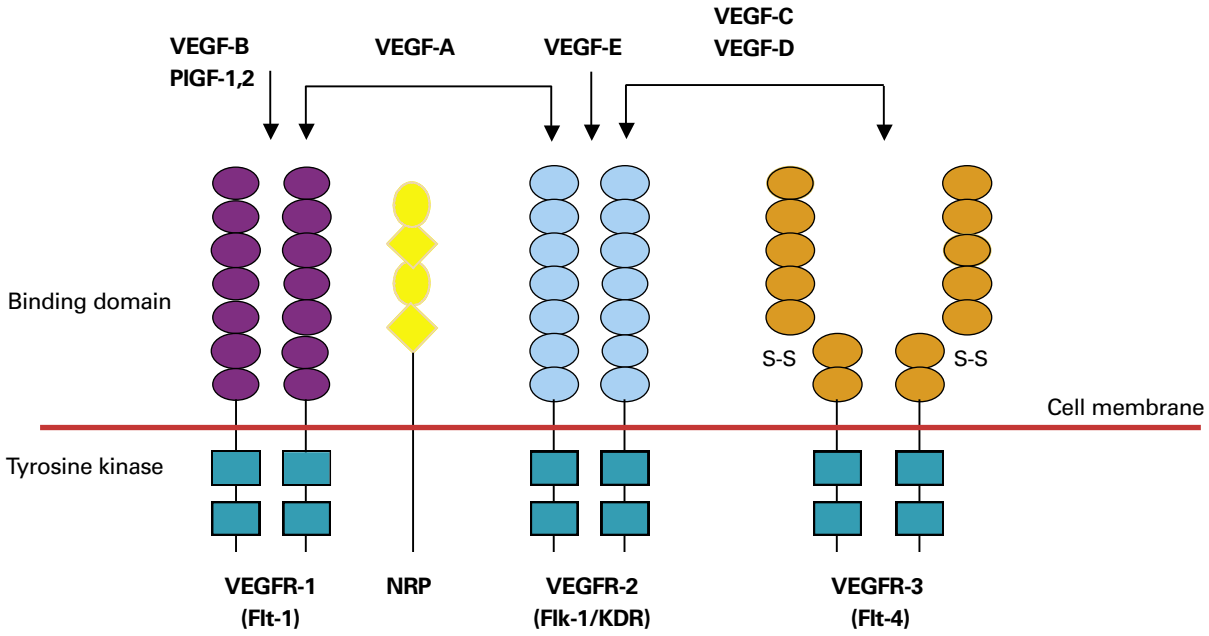
Interferon-alfa has an anti-angiogenic effect by inhibiting endothelial cell migration. It has been successfully used to treat haemagiomas, refractory giant cell tumours and angioblastomas.

Thalidomide

There has been renewed interest in this potent teratogen since it has been shown to be both an immunomodulatory and

Fig. 1

Vascular endothelial growth factor receptors [2]



The vascular endothelial growth factor receptors (VEGFR) consist of a binding domain and a tyrosine kinase domain. Each receptor is associated with a different form of tyrosine kinase (Flt-1, Flk-1/KDR, Flt-4). The neuropilin receptors (NRP) act as co-receptors for vascular endothelial growth factor (VEGF). Some of the VEGF molecules bind with more than one receptor. Placental growth factor (PlGF) binds with VEGFR-1.

anti-angiogenic drug. Thalidomide is thought to inhibit angiogenesis by reducing levels of bFGF, VEGF, COX-2 and tumour necrosis factor (TNF-α). It may also reduce tumour-induced overproduction of circulating precursors of endothelial cells.

In patients with multiple myeloma there is an increased rate of angiogenesis within the bone marrow. Thalidomide has been used in the treatment of resistant multiple myeloma as it has anti-angiogenic effects and can directly inhibit the growth and survival of myeloma cells.

VEGF inhibitors

Inhibition of the VEGF pathway has become the focus of angiogenesis research as approximately 60% of malignant tumours express high concentrations of VEGF. Strategies to inhibit the VEGF pathway include antibodies directed against VEGF or VEGFR, soluble VEGFR/VEGFR hybrids, soluble analogues of the VEGFR (VEGF-Trap) and tyrosine kinase inhibitors. One of the earliest strategies to inhibit VEGF activity involved the use of antibodies directed against VEGFRs. For example, preclinical data with anti-VEGFR-2 antibodies demonstrated decreased VEGF-induced signalling, decreased angiogenesis and decreased primary and metastatic growth in a variety of tumour systems.

VEGF-Trap is a decoy receptor. It consists of parts of VEGFR-1, VEGFR-2 and immunoglobulin G (IgG). The molecule is soluble and binds to VEGF-A before it can reach its normal receptors. VEGF-Trap binds VEGF-A 100- to 1000-fold more tightly than monoclonal antibodies. It inactivates all circulating and tissue VEGF-A isoforms and PlGF.

Several small molecule inhibitors of tyrosine kinase activity have been developed. These have activity not only against VEGFR-2, but also on other VEGFRs, fibroblast growth factor receptor, the epidermal growth factor receptors (EGFR*) and platelet derived growth factor receptors (PDGFR-α, PDGFR-β). For example, sunitinib (SU11248) has activity against VEGFR-2 and PDGFR (see Table 1).

Bevacizumab

Bevacizumab is derived from a monoclonal antibody to murine VEGF. Genetic engineering produces a 93% human and 7% murine protein sequence. The molecule has the same biochemical and pharmacologic properties as the natural antibody, but with reduced immunogenicity and a longer biological half-life. By binding to VEGF-A bevacizumab prevents it from binding with its receptors.

Preclinical studies reported impressive responses and prevention of tumour growth in almost all tumour xenografts. Bevacizumab has been studied in a number of clinical trials and is approved for use in metastatic colon cancer.

* EGFR is also known as human epidermal growth factor receptor (HER) and these abbreviations are interchangeable.

Other strategies

There are a variety of other drugs that have at least some anti-angiogenic properties. It has been known for some time that low-dose chemotherapy with cytotoxic drugs, such as the taxoids, produces some anti-angiogenic effects. In addition, inhibition of other molecular targets also has the potential to interfere with angiogenesis. These targets include EGFR, COX-2, TGF-α and the proteosome. Other drugs that have been shown to have anti-angiogenic effects in preclinical models are zoledronic acid and rosiglitazone.

COX-2 is an important mediator of angiogenesis and tumour growth. COX-2 expression occurs in a wide range of preneoplastic and malignant conditions. The enzyme has been localised to neoplastic cells, endothelial cells, immune cells, and stromal fibroblasts within tumours. It mediates its pro-angiogenic effects primarily by three products of arachidonic acid metabolism – thromboxane A_2, prostaglandin E_2 and prostaglandin I_2. These products promote angiogenesis by a number of mechanisms including stimulation of VEGF, promotion of vascular sprouting and tube formation, increased survival of endothelial cells and activation of EGFR-mediated angiogenesis. Studies have shown that selective inhibition of COX-2 activity will suppress angiogenesis *in vitro* and *in vivo* and therefore COX-2 inhibitors could be a useful adjunct to therapy.

Expression of human epidermal growth factor receptor 2 (HER-2) within tumour cells is closely associated with angiogenesis and VEGF expression. This is thought to be mediated by transregulation of HER-2 by proteins called heregulins. These heregulins regulate the expression and secretion of VEGF in breast cancer cells. Trastuzumab is a monoclonal antibody that blocks HER-2.[1] This reduces tumour cell growth and VEGF expression by the inhibition of heregulin-mediated angiogenesis both *in vitro* and *in vivo*. Trastuzumab is currently available for patients with metastatic breast cancer if the tumour overexpresses HER-2.

It is not known how much of the anticancer effects of drugs aimed at molecular structures are due to their angiogenic effects. Angiogenesis is a complex process and successful inhibition of angiogenesis may involve the combination of multiple drugs with differing modes of action.

Another strategy related to angiogenesis is the destruction of new vessels. This has led to the development of vascular targeting drugs (Table 1).

Adverse effects

The full spectrum and aetiology of toxicities produced by the angiogenesis inhibitors has yet to be defined. The induction of venous and arterial thromboses, bleeding, hypertension and proteinuria by drugs, such as bevacizumab, is probably directly related to their effects on endothelial cells. The gut perforation occasionally associated with bevacizumab may be related to induction of ischaemia. The teratogenic effects of thalidomide may have been due to its action on peripheral blood vessel development in the fetus. However, it is unlikely that the common adverse effects of thalidomide such as somnolence, rash and neuropathy are related to its effect on angiogenesis.

Conclusion

The use of angiogenesis inhibitors is an exciting new area of cancer research. Their optimal use has yet to be defined.

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Note: This article has intentionally not been fully referenced. The following articles are recommended for further reading on the topic.

Further reading

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Stephen Clarke is the Principal investigator for Asia Pacific for two Roche-sponsored studies involving bevacizumab. He has been a member of a Roche colorectal advisory board and an invited speaker at Roche-sponsored clinical meetings.

Self-test questions

The following statements are either true or false (answers on page 27)

3. Increased expression of angiogenic factors is associated with an improved prognosis for patients with cancer.
4. Bevacizumab may cause thrombosis and haemorrhage.