Inhibition of angiogenesis and the angiogenesis/invasion shift

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Abstract
Angiogenesis has become a major target in cancer therapy. However, current therapeutic strategies have their limitations and raise several problems. In most tumours, anti-angiogenesis treatment targeting VEGF (vascular endothelial growth factor) has only limited overall survival benefit compared with conventional chemotherapy alone, and reveals several specific forms of resistance to anti-VEGF treatment. There is growing evidence that anti-VEGF treatment may induce tumour cell invasion by selecting highly invasive tumour cells or hypoxia-resistant cells, or by up-regulating angiogenic alternative pathways such as FGFs (fibroblast growth factors) or genes triggering new invasive programmes. We have identified new genes up-regulated during glioma growth on the chick CAM (chorioallantoic membrane). Our results indicate that anti-angiogenesis treatment in the experimental glioma model drives expression of critical genes which relate to disease aggressiveness in glioblastoma patients. We have identified a molecular mechanism in tumour cells that allows the switch from an angiogenic to invasive programme. Furthermore, we are focusing our research on alternative inhibitors that act, in part, independently of VEGF. These are endogenous molecules that play a role in the control of tumour growth and may constitute a starting point for further development of novel therapeutic or diagnostic tools.

Introduction
The formation of new blood vessels (angiogenesis) is essential for embryonic development, postnatal growth and wound healing. It also contributes significantly to pathological conditions. Insufficient angiogenesis leads to tissue ischaemia (e.g. ischaemic heart disease or stroke), whereas excessive vascular growth promotes cancer, chronic inflammatory disorders (e.g. arthritis or psoriasis) or ocular neovascular diseases [1]. All of these disorders constitute major causes of morbidity and mortality in Western societies. Algire and Chalkley in 1945 [1a] and Folkman in the 1970s [2] suggested that blocking the blood supply of a tumour might represent a novel therapeutic strategy to inhibit tumour growth. Over the subsequent decades, researchers have gained essential insight into the biological and molecular mechanisms of angiogenic vessel growth. In this context, significant progress has been made to determine how proliferating ‘angiogenic’ endothelial cells differ from their non-proliferating ‘quiescent’ counterparts and how their survival, growth and remodelling is controlled. This has led to the development of a number of therapies aimed at modulating vessel growth in patients with angiogenesis-related diseases [3].

Angiogenesis inhibition has generated positive results in clinical trials in colon, kidney, lung and breast cancer. The discovery of a link between the VHL (von Hippel–Lindau) tumour-suppressor gene, HIF-1 (hypoxia-inducible factor 1), and VEGF (vascular endothelial growth factor) in tumour growth has identified a pathway for targeted therapy in renal cancer [4]. Furthermore, VEGF itself has emerged as a critical driver of angiogenesis in colorectal cancer, breast cancer and NSCLC (non-small-cell lung carcinoma). Several strategies targeting these pathways have been investigated in clinical studies. New agents including the small kinase inhibitors sunitinib (Sutent™, Pfizer), sorafenib ( Nexavar™, Bayer/Onyx) and temsirolimus ( Torisel™, Wyeth), and the monoclonal antibody bevacizumab ( Avastin™, Wyeth), have shown significant anti-tumour activity and provided survival benefits in randomized clinical trials. Therapies integrating these drugs have now become the standard of care for advanced/metastatic kidney, breast and colorectal cancers and NSCLC [5].

Resistance to anti-angiogenesis therapy
In spite of this undeniable success, current anti-angiogenic therapies have revealed some limitations, and several unanticipated problems are emerging. First, in most tumours (with the exception of renal cell carcinoma), anti-angiogenesis treatment requires association with chemotherapy. Secondly, in most patients, addition of anti-angiogenic therapy to current standard therapies provides only limited overall survival benefits. Thirdly, an unexpected problem of anti-angiogenesis therapy in cancer is the development of resistance. This seems to be common to all currently

Key words: angiogenesis, chemokine, combinatorial therapy, resistance to anti-angiogenesis therapy, stress response, vascular endothelial growth factor (VEGF).

Abbreviations used: CAM, chorioallantoic membrane; CXCL4, CXC chemokine ligand 4; CXCL4L1, CXCL4-like 1; GBM, glioblastoma multiforme; IL, interleukin; IRE1, inositol-requiring enzyme 1; NSCLC, non-small-cell lung carcinoma; UPR, unfolded protein response; VEGF, vascular endothelial growth factor.

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Combination of anti-VEGF therapy and IL (interleukin)-6 blockade

For this purpose, we investigated whether combined inhibition of the VEGF and the IL-6 pathways would be beneficial and enhance therapeutic efficacy.

GBM (glioblastoma multiforme) is the most aggressive brain tumour and among the deadliest cancers. No effective therapies for GBM exist to date. GBM cells are characterized by their invasive abilities and a striking angiogenic potential, which distinguishes them from low-grade glioma. Ideally, anti-glioma therapy should take into account the up-regulation of various molecular pathways that contribute to malignancy progression including invasion and induction of angiogenesis.

The anti-VEGF antibody Avastin™ (Roche) is currently used in the clinic for GBM. We and others reported that IL-6 is abundantly produced by glioma cells and contributes to malignancy by promoting angiogenesis, cell proliferation and resistance to apoptosis [11,12]. Inhibition of IL-6 and VEGF leads to inhibition of U87-derived experimental glioma grown on the chick CAM (chorioallantoic membrane) or in the brain of xenografted mice [12]. Combined IL-6/VEGF knockdown not only showed enhanced reduction of tumour growth and angiogenesis, but also significantly prevented invasion of residual tumour cells (Figure 1). In mice, combining IL-6 knockdown and Avastin™ treatment completely abrogated tumour development and infiltration [12]. We have recently validated these results further by combining Avastin with Actemra (Roche), an anti-(IL-6 receptor) antibody already used in the clinic for rheumatoid arthritis patients. The two drugs together are significantly more efficient than Avastin alone, both in in vitro angiogenesis assays and in xenografted mice (S. Javerzat, M. Hagedorn and A. Bikfalvi, unpublished work). Furthermore, transcriptomic/proteomic analysis of treated tumours show a reduced activity of major pathways for cell survival, proliferation and invasiveness in remaining tumour cells that are still active after treatment with VEGF or IL-6 inhibitors alone.

The stress response, a critical sensor for tumour angiogenesis/invasion shift

Several general mechanisms of adaptive/evasive resistance of tumour cells to antiangiogenesis treatment have been described, including up-regulation of alternative growth factors or receptors, or infiltration of mononuclear cells.

clinically used anti-angiogenic drugs. Fourthly, there is mounting evidence suggesting that anti-VEGF therapy (or anti-angiogenic therapy at large) may paradoxically enhance tumour progression by promoting an invasive phenotype that allows the tumour to escape angiogenesis inhibition. Experimental models reproducing evasive resistance, i.e. adaptation to circumvent the specific angiogenic blockade, have recently shown that escape from anti-angiogenesis is also associated with increase in metastatic tumour cell dissemination at lymph nodes and distant sites [6–8].

Different mechanisms of evasive and intrinsic resistance to anti-VEGF therapies have been proposed including up-regulation and use of alternative pathways such as FGFs (fibroblast growth factors), selection of hypoxia-resistant cells and/or induction of genes triggering invasive programmes [9]. Interestingly, in experimental mouse models, pre-treatment with anti-VEGF antibodies before tumour cell implantation will prime the host tissue to promote pro-invasive programmes in tumour cells, suggesting that anti-VEGF therapy may induce a systemic adaptive response in the healthy tissue favouring tumour progression [8]. In this regard, the role of BMDCs (bone-marrow-derived cells) in the acquisition of resistance to anti-VEGF treatment has been recently shown to promote the infiltration of tumours by CD11b+ myelomonocytic cells [10]. Taken together, these observations underscore the need to understand how tumours react and adapt to anti-angiogenic therapies, in particular those targeting the VEGF pathway, and to identify new targets to develop alternative therapeutic strategies and eventually overcome resistance.

Figure 1 | Combined inhibition of VEGF and IL-6 in the CAM model

U87 glioma cells were implanted into the CAM where either VEGF or IL-6, or both, was inhibited using siRNA (small interfering RNA). As can be seen, combined inhibition significantly inhibited tumour cell invasion into the surrounding stroma. NC, negative control. Invasion was scored as either individually (Ind) or collectively (Coll) migrating. Results are means ± S.E.M. *P < 0.05, ***P < 0.001 (Kruskal-Wallis test). Reprinted from [12] with permission.
However, the precise mechanisms, at the level of the tumour cell, are not understood.

Ischaemia is associated with tumour development and locally induces an adaptive response, which confers on tumour cells an enhanced survival and a more aggressive behaviour. A better knowledge of tumour responses to ischaemia is required to elaborate therapeutic strategies of cell sensibilization and angiogenesis inhibition, based on the blockade of survival mechanisms. IRE1 (inositol-requiring enzyme 1), one of the three proximal sensors of the UPR (unfolded protein response), is a key regulator of these effects [13]. We have previously identified IRE1 as an upstream key regulator of the tumour angiogenesis process and have given support to the idea that the IRE1 branch of the UPR plays a major role in cancer development [14,15]. We have recently shown that, in a glioma model, the blockade of IRE1 signalling was correlated with down-regulation of prevalent pro-angiogenic factors such as VEGF-A, IL-1β, IL-6 and IL-8 [16]. Significant up-regulation of several anti-angiogenic gene transcripts was also apparent, which included SPARC (secreted protein acidic and rich in cysteine), decorin and thrombospondin-1. Abrogating the activity of the central sensor IRE1α strongly inhibits tumour angiogenesis, but also increases the invasive behaviour of glioma cells in the normal brain parenchyma [14,16] (Figure 2). This phenotype resembles that of the cellular response of anti-angiogenesis resistant tumours [6,8]. From these experiments, it became apparent that IRE1 inhibition causes both pro- and anti-tumorigenic effects.

‘Old’ new players: CXC chemokines

VEGF family members, angiopoietins, Notch/Delta4 or platelet-derived growth factors are, at present, the major focus of angiogenesis research. However, there are many other regulatory molecules, such as the chemokines, able to critically modulate vessel growth.

Chemokines are broad-range regulators that play important roles in development, inflammation, HIV pathophysiology and cancer [17]. They are divided into four subfamilies, based on structural properties and primary amino acid sequence, as CXC, CC, C or CX3C. CXC chemokines represent a large family of homologue peptides exhibiting positive or negative activity on the control of angiogenesis. Angiostatic CXC chemokines have an important role against tumour development and diffusion. As these molecules also directly affect the tumour cells themselves, they may be, in addition, involved in adaptive/evasive resistance.

Overexpression of CXCL4 (CXC chemokine ligand 4) and IP-10 (interferon-γ-inducible protein 10) blocks tumour progression and can also induce regression of metastasis. Our laboratory and collaborators have extensively contributed in the study of CXCL4 [18–23]. We have demonstrated anti-angiogenic, anti-invasive and anti-tumour properties of a C-terminal fragment of CXCL4 (CXCL4CTF). In addition, we have partially elucidated its interaction with angiogenic growth factors and integrins and identified the critical amino acids for these interactions.

The discovery of a previously unrecognized alternatively spliced variant of the CXCR3 receptor called CXCR3-B, (the already known variant was renamed CXCR3-A), has allowed a better understanding of how CXC chemokine activity is exerted [24]. CXCL4L1 (CXCL4-like 1), an ELR (Glu-Leu-Arg)-negative chemokine, has been described as strongly inhibiting angiogenesis [25]. Mature CXCL4L1 is highly homologous with CXCL4 and only differs by three amino acids. These differences, however, have considerable functional importance: the anti-angiogenic activity of CXCL4L1 is increased 43–500-fold compared with CXCL4. We have studied the mechanism of action of CXCL4L1 and demonstrated unique features of this molecule [26,27]. CXCL4L1 binds proteoglycans to a much lesser extent and is much more diffusible than CXCL4 [27] (Figure 3). Furthermore, we have demonstrated specific overexpression of CXCL4L1 in several solid tumours (C. Quemener, A. Dubrac, H. Prats and A. Bikfalvi, unpublished work).

In a previous study, changes in CXCL4 were detected across a spectrum of human cancers in mice [28]. SELDI-TOF
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Figure 3 | Surface plasmon resonance experiments with recombinant CXCL4L1 (rCXCL4L1) in comparison with recombinant CXCL4 (rCXCL4)

As can be seen, binding of CXCL4L1 to heparin and heparan sulfate is much weaker than for CXCL4. Furthermore, no binding to chondroitin sulfate is seen for CXCL4L1 in contrast with CXCL4. RU, response units. Reprinted from [27] with permission.

(surface-enhanced laser-desorption ionization–time-of-flight) MS and tandem MS together with ProteinChip immunoassay identified platelet-associated CXCL4 and possibly CXCL4L1 up-regulation in mice in various xenografted human tumours. This suggests that CXCL4 chemokines and/or CXCL4L1 are potential markers for diagnosis and prognosis of malignant disease in humans.

Taken together, these results indicate that CXCL4L1 may represent a novel and interesting molecule for both therapeutic and diagnostic purposes in angiogenesis-related diseases. This may have a significant impact for the management of angiogenesis-resistant tumours.

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