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Drug Resistance Associated with Antiangiogenesis Therapy

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Abstract

Neovascularization is one of the hallmarks associated with tumor growth. In the recent years, a number of angiogenesis inhibitors have been approved for clinical use in cancer patients. However, the efficacy of antiangiogenic therapy is in most cases short-lasting, with likely drug resistance developing within a few months. It is becoming clear also that there are a subset of malignant tumors that are inherently resistant to angiogenesis inhibition. The knowledge regarding resistance mechanisms towards angiogenesis inhibitors is still evolving and here we propose some theories and in some cases provide experimental evidence.

Keywords

cancer; angiogenesis; antiangiogenic therapy; drug resistance mechanisms

Introduction

Since the seminal articles about the importance of tumor angiogenesis and the possibility of antiangiogenesis therapies in the early 1970's [1,2], the field of angiogenesis has expanded to become one of the major areas of cancer research today. Antiangiogenic drugs are currently approved by FDA for clinical use, in breast cancer, colorectal cancer, lung cancer and renal cell carcinoma, but only in patients with metastatic disease and without a curative potential [3-8]. Except for patients with renal cell carcinoma, the angiogenesis inhibitors have to be combined with chemotherapy in order to obtain any significant tumor response.

In the late 1990's, the curative potential of antiangiogenic therapy was warmly debated in the scientific community [9,10]. Theoretically, it was proposed that targeting the tumor

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endothelium would circumvent the problem of acquired drug resistance [11]. Experimentally, the initial studies indicated that drug resistance would not emerge with antiangiogenic therapy [12,13], but there are now a large number of studies which demonstrate that treatment failure is a common finding in animal models when angiogenesis inhibitors are given as a monotherapy [14-16]. Also the results of all clinical trials conducted with angiogenesis inhibitors to date show that drug resistance develops within a few months of the commencement of antiangiogenic therapy. Here we will discuss various experimental concepts that may explain resistance to angiogenesis inhibitors and in some cases provide scientific data that demonstrates such resistance mechanisms.

Mutations in Endothelial Cells

The neovasculature of a growing malignancy develops due to endothelial cells that are recruited from the surrounding normal tissues. This is demonstrated by genetic tracing of host endothelial cells by implanting a LacZ negative tumor in mice that are Tie2-LacZ positive [17] or by growing dsRed-labelled tumors in mice expressing enhanced green fluorescent protein (eGFP) in all normal tissues [18] (Fig. 1). While the unstable genome of the cancer cells commonly cause them to develop acquired drug resistance, the tumor endothelium presumably have a normal genome and lack the ability to circumvent drug inhibition [11,19,20]. This is supported by a number of experimental studies wherein angiogenesis inhibitors have been administered over long time periods without developing drug resistance [13,21,22].

There are a few publications suggesting that the tumor endothelium may also harbor genetic aberrations in non-endothelial cancers [23-26]. In the article by Hida et al., endothelial cells isolated from human tumors grown in nude mice frequently have an abnormal karyotype with excessive number of centrosomes [23]. The probability of human tumor cell contamination in the analyzed mouse endothelial cultures is low as the human-specific difteria toxin was used to clear the cultures of human cells, and also, mouse-specific chromosomal probes were used for *in situ* hybridization analysis. In the same article, heterogenous chromosomal rearrangements in the endothelial cells indicate that the genetic alterations are non-clonal and probably a result of the permissive microenvironment within malignant tumors, or an *in vitro* phenomenon. Furthermore, the extent of aneuploidy increased upon passaging of tumor endothelial cells *in vitro*, compared to normal tissue endothelial cells, indicating an inherently unstable genome within the tumor endothelium [24]. In another article by the same group they show that in a spontaneous prostate cancer developing in mice, the tumor endothelium does not carry the SV40 T antigen that causes cancer development in the prostate epithelial cells [27].

In the article by Gunsilius et al., patients with chronic myelogenous leukemia reveal the BCR/ABL fusion gene within normal endothelial cells in the myocardium and in bone marrow-derived endothelial cells cultured *in vitro* [26]. The reason for mutations in the endothelium is likely due to a common bone marrow progenitor that harbors the fusion gene, and forwards it both to the leukemia cells and to circulating endothelial cells that are incorporated in normal tissue blood vessels [26].

In an analysis of tumor specimens from patients with B-cell lymphomas, lymphoma-specific chromosomal translocations were found in tumor endothelial cells [25]. The identification of endothelial cells was performed by immunostaining for CD31, von Willebrand factor or *Ulex europaeus* lectin. However, only 37% of the tumor endothelial cells were found to harbor chromosomal alterations. Given the inherent potential for non-specific immunoreactivity in antibody labelling experiments, there is a risk that some of the identified “endothelial” cells could be lymphoma cells instead.

However, if the findings in the above articles are indeed correct, the impact of antiangiogenic therapy could be far more limited then anticipated because drug resistance could develop due to evolving genomic instability of the tumor endothelium. Also, if tumor endothelial cells do indeed possess an unstable genome, it could explain why tumor endothelial cells in culture are more resistant to vincristine than normal endothelial cells [28]. All in all, the data supporting genetic alterations in the tumor endothelium is limited, and more research is needed in this area.

Angiogenic Signaling Redundancy - When One Factor is Blocked, Another is Upregulated

In malignant cancer cells multiple signaling pathways are commonly dysregulated at the same time [29-31]. Moreover, distinct malignant cell clones may have different signaling pathways dysregulated, as demonstrated in acute myeloid leukemia [32]. This implies that redundancy is to be expected when targeting a single signaling pathway, as other pathways are likely to go unchecked and could compensate for the one being targeted (Fig. 2).

If tumor growth is inhibited by overexpressing thrombospondin-1 (TSP-1), endostatin or tumstatin, these tumors finally escape and progress due to upregulation of angiogenic growth factors, in particular vascular endothelial growth factor (VEGF) [14]. Anti-VEGF receptor-2 (VEGFR-2) treatment in Rip1-Tag2 pancreatic tumors provides a transitory tumor response, followed by tumor regrowth with upregulation of fibroblast growth factors 1 and 2 (FGF1 and FGF2), ephrin A1 and A2, as well as angiopoietin-1 [33]. Such evasive resistance to anti-VEGFR-2 treatment could be suppressed by commencing therapy with FGF-trap to block FGF1 and FGF2 signaling [34]. Similarily, when a pan-VEGFR inhibitor was used in patients with glioblastoma multiforme, those that failed on therapy were found to have increased levels of FGF2, stromal cell-derived factor (SDF)-1 and circulating endothelial cells [35]. Experimentally, c-Met, platelet derived growth factor receptor (PDGFR)-α and epidermal growth factor receptor (EGFR) were all found to converge on and activate the phosphatidylinositol-3-kinase (PI3K) pathway in glioblastoma, lung and pancreatic tumor cell lines [36], indicating that such one-axis inhibition will be insufficient. In accordance with these preclinical data, signal transduction inhibitors targeting either EGFR or PDGFR were found to be ineffective in glioblastoma patients, and by using an antibody array the tumor samples were found to have multiple receptor tyrosine kinases (RTKs) upregulated simultaneously [36]. In another study signaling molecules such as extracellular signal-regulated kinase (ERK), Akt, mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription protein 3 (STAT3) were all found to be activated in patients with pancreatic ductal adenocarcinoma [30].

It is difficult to interpret from the above studies whether the signaling redundancy is from within the malignant cell population or the supporting stroma or endothelium. However, heterogeneity is evident when endothelial cells from different normal organs and different tumor types are compared [37,38]. Moreover, the tumor endothelium differs from the surrounding normal tissue endothelial cells by having a multitude of genes upregulated [20,28], indicating that targeting a single cytokine or growth factor has a low probability of long-term therapeutic success. In a melanoma xenograft model, EGFR expression was found mainly on the tumor endothelial cells, and EGFR inhibition by gefitinib led to tumor growth inhibition, but subsequent upregulation of VEGFR-2 on the endothelium [39]. Thus, the addition of anti-VEGFR-2 to gefitinib treatment would be an appropriate strategy in this particular case.

In the VEGF/VEGFR family system, multiple ligands are encountered, with overlapping receptor specificities. For instance, when VEGF (VEGF-A) is blocked, VEGF-C and VEGF-D can take over its signaling function [40]. A feasible strategy to avoid such a resistance mechanism is the use of soluble VEGF receptors. Whereas antibodies directed at one specific VEGF receptor can be circumvented by ligand binding to other VEGF receptors, the administration of soluble VEGF receptor will effectively prevent such binding by absorbing the ligands [41]. Apart from the VEGF family, another example of crosstalk is between prostaglandin E2 and transforming growth factor β (TGFβ), both activating the activin-like kinase 5 (Alk5) receptor to stimulate angiogenesis [42].

Using cocktails of different angiogenesis inhibitors is a viable strategy to prevent signaling redundancy in tumor associated endothelial cells [34,41]. After transfecting tumor cells with angiostatin and endostatin, a synergistic antiangiogenic activity was found in both leukemia and melanomas in mice [43]. In human renal cell carcinoma xenografts, the combination of anti-VEGF and the endogenous tumstatin peptide gave a significant tumor growth delay, whereas each of the compounds individually yielded marginal tumor response [44].

The dependency of tumors on a particular angiogenic growth factor can change during antiangiogenic therapy. It has been shown that during prolonged anti-VEGF therapy, vascular remodeling occurs where tumor blood vessels get increased pericyte coverage due to upregulation of PDGF-B and ephrin B2 [45,46]. When these blood vessels acquire pericytes their dependency on VEGF decreases and they turn resistant to anti-VEGF therapy [46,47]. By using a combined inhibition of VEGF and PDGF receptors, one can circumvent this problem and enforce tumor vessel regression [48]. On the other hand absence of α-smooth muscle actin (αSMA) positive cells around the blood vessels correlates with hematogenous metastasis and poor prognosis in colorectal cancer patients [49], indicating that pericyte coverage on the tumor vasculature may protect against metastasis. There is in fact increasing evidence that a combined targeting of tumor endothelium and pericytes by combined VEGFR and PDGFR blockage facilitates the metastasis process [34].

Direct Versus Indirect Angiogenesis Inhibitors

Angiogenesis inhibitors can be divided into two groups, the direct and indirect angiogenesis inhibitors. Direct angiogenesis inhibitors target the endothelial cell behavior directly,

whereas indirect inhibitors target cytokines or growth factors acting upon endothelial cells [50]. Theoretically, acquired drug resistance is expected to be a significant problem when indirect angiogenesis inhibitors, such as anti-VEGF antibodies are administered, as the tumor endothelial cell could still be stimulated by other growth factors that are not inhibited. Angiogenic growth factors are commonly produced by the tumor cells themselves, and the tumor cell population could easily evade such growth factor inhibition by shifting their signaling dependency. In human squamous cell carcinoma xenografts, anti-EGFR treatment initially downregulates VEGF production, but after two weeks of continous therapy, some tumor cell clones expand that continue to secrete VEGF despite anti-EGFR treatment [51].

Direct inhibitors, on the other hand, target signaling pathways within the endothelial cell, inducing apoptosis or inhibiting cell migration (Table 1). Endogenous angiogenesis inhibitors seem to exert direct endothelial cytotoxicity as a common characteristic. Apart from tumor cell cytotoxicity, several types of chemotherapeutic agents like cyclophosphamide and combretastatin A-4, also exhibit a direct cytotoxic effect on tumor endothelium [52-54]. Direct angiogenesis inhibitors in many cases actively induce endothelial cell apoptosis, therefore persistant signaling through other signaling pathways may not rescue the cell. Thus, the addition of direct inhibitors to indirect angiogenesis inhibitors as a therapy cocktail is likely to improve the therapeutic outcome [44,55].

Tumors Can Grow Without Blood Vessels

It is generally thought that a tumor cannot grow beyond 2-3 mm without the recruitment of neovasculature [1]. Furthermore, some experiments have demonstrated that angiogenesis is initiated even in carcinomas *in situ* [72,73] and also in tumor nodules as small as 0.1 mm [74]. However, the notion that tumors require blood vessels in order to expand beyond 0.1 mm is not an absolute concept, according to some investigators. These studies propose that there are various methods by which cancer cells can grow without the recruitment of new blood vessels.

In glioblastoma multiforme, it has been known for a long time that cancer cells can migrate as diffuse colonies into the surrounding brain, without initiating angiogenesis [75]. Additionally, cancer cells can adapt to a hypoxic environment when tumor angiogenesis is inhibited, by selection of clones that are p53 negative and therefore become hypoxia-resistant [76]. Upon anti-VEGF or anti-VEGFR treatment, glioma cells in some cases are found to react by “vascular cooption” [77-79]. Vascular cooption is a term used when tumor cells grow around the pre-existing blood vessels in the normal tissue, receiving all the required nutrients and oxygen for further proliferation without the need for recruiting a new vasculature (Fig. 3). This phenomenon has been described both in gliomas and lung cancer [80-82].

Increased Vessel Density Without the Need for Endothelial Proliferation

Sprouting angiogenesis is a sequential process wherein endothelial cells proliferate, migrate and mature into new vessels. However, blood vessels can also split into new vessels without the need for endothelial proliferation, and this process is termed intussusception [83]. Intussusception is demonstrated in various types of malignant tumors, and has been

suggested to occur in the absence of VEGF, as a fast and energy-conserving way of developing new tumor vessels [83]. Clinically, accumulations of tumor blood vessels generated by intussusceptive vessel growth correlate with a worse patient outcome for various types of cancers [84]. While anti-proliferative endothelial inhibitors (such as anti-VEGF antibodies) stop sprouting angiogenesis, intussusceptive vessel growth would probably not be affected by these inhibitors. Instead anti-migratory agents might be more effective in this setting.

Vasculogenic Mimicry

The target of antiangiogenic therapy is generally the endothelium within malignant tumors. Therefore, if tumor cells can develop blood conducting channels without the need for endothelial cells (i.e. vasculogenic mimicry), antiangiogenic treatment would potentially fail. Debates about the validity of vasculogenic mimicry within tumors still continue [85-87]. Some investigators express concerns regarding the interpretation of vasculogenic mimicry [87]. Tumor cells lining the vessel lumen could be due to apoptosis of the overlying endothelial cells, or simply a loss of endothelium *ex vivo* due to tissue preparation or cutting artefacts. Furthermore, they could be tumor cells in the process of invasion via the blood stream to metastasize. On the other hand, there is compelling evidence that vasculogenic mimicry is encoutered in some tumor types.

There are articles describing vasculogenic mimicry in malignant melanoma, sarcoma, glioma, breast cancer and many other cancer types [83,88-90]. It has been found that melanoma cells can dedifferentiate and display a vascular phenotype, induced by an ischemic microenvironment [86,91]. When analyzing erythrocyte-containing channels in intraocular melanoma tissue from cancer patients, endothelial cells in some tumors were not found by light microscopy, transmission electron microscopy or immunohistochemical staining [89]. In patients with choroidal melanomas these channels were found to be functional, i.e. perfused by blood [83]. In dsRed-expressing U87 glioma xenografts grown in eGFP mice, erythrocyte-containing channels were detected without an endothelial cell lining [18]. Also, in breast carcinoma xenografts, vascular channels composed of tumor cells were found to be perfused with blood when evaluated by MRI angiography, and tumor cells lining the channels expressed endothelial markers such as Flt-1 and Tie-2, but not CD31 [90]. However such data is not present for *spontaneous* mouse tumors. As a variant of vasculogenic mimicry, *mosaic vessels*, wherein a mixture of cancer cells and endothelial cells line the vessel wall, has been identified in various tumor types [92]. In this study, about 15% of the perfused tumor blood vessels show a mosaic pattern, including human tumors. How these type of vessels respond to anti-angiogenic therapy is still unknown.

Heterogenous Dependence of Tumors on Angiogenic Growth Factors

There are a large number of endogenous angiogenic and antiangiogenic factors identified as of today [83,93], and individual differences exist. Different mouse strains exhibit a distinct angiogenic response to FGF1 and VEGF [94]. Also, various mouse strains differed in their angiogenic response to FGF1 and VEGF when recruitment of circulating endothelial cells to the tumors are used as a read out [95].

Furthermore, different cancer types probably produce and depend on different angiogenic growth factors. This is suggested by preclinical data showing that anti-VEGF treatment was much less effective in neuroblastoma than Wilms tumor xenografts [96]. Therefore even if a drug is effective against one growth factor, the therapy can still fail if this factor is not important for the endothelium in that given tumor [37]. Also, the maturation of the vasculature within a particular tumor may determine the effectiveness of angiogenesis inhibition, for instance VEGF functions as a survival factor only in blood vessels that are not covered by pericytes [97].

Clinically, there are clear differences between different cancer types with respect to sensitivity towards antiangiogenic treatment [34]. While anti-VEGF monotherapy significantly inhibits the growth of renal cell carcinomas, the effect in many other tumor types is negligable when not combined with chemotherapy [98,99]. Furthermore, there are obvious differences between cancer patients with respect to their response to angiogenic stimulation. For example, about 20% of patients with metastatic renal cell carcinoma do not respond to anti-VEGF treatment [98,100]. On the other hand, there are a small subset of patients with metastatic renal cancer with impressive response to antiangiogenic therapy, with disease stabilization observed for 3-5 years [101]. Clearly, tumor tissue from these good responders will likely provide clues to the positive response.

Non-Endothelial Targets of Antiangiogenic Therapy

A malignant tumor consists of many different cell types, including tumor cells, endothelial cells, fibroblasts, macrophages and other leukocyte subpopulations. These cells are important contributors to the production of angiogenic growth factors as well as other protumorigenic cytokines [34,93]. If endothelial-specific inhibitors are used as antiangiogenic agents, the other cells in the tumors remain capable of producing angiogenic growth factors [34] (Fig. 4). One example of such a scenario is the use of VEGFR-2 signaling inhibitors. When VEGFR-2 is blocked on endothelial cells, fibroblasts and tumor cells can still secrete VEGF and stimulate the endothelium via VEGFR1 and VEGFR3 [102]. Moreover, tumors may evade antiangiogenic therapy by secreting SDF-1 from tumor-associated fibroblasts to attract circulating endothelial precursor cells and continue the process of neo-vascularization [103]. Macrophages seem to accumulate in hypoxic tumor areas and via production of hypoxia-inducible factor-1α (HIF1α) and VEGF contribute to tumor angiogenesis [104]. Furthermore, during pancreatic islet carcinogenesis, infiltrating neutrophils are important contributors when preneoplastic lesions switch to an angiogenic and invasive cancer phenotype [105].

The Effective Dosage of Antiangiogenesis Agents Used in the Clinic

The traditional way of treating cancer patients has been to administer the maximal tolerated dose (MTD), with the bone marrow side effects often used as a limiting factor. After bone marrow recovery, repeated doses are given, commonly every three weeks, and the treatment is continued usually for 3-12 months. When angiogenesis inhibitors are used for cancer therapy, the MTD dosage schedule is not clearly defined.

Endostatin exposure of endothelial cells *in vitro* yieldes a U-shaped dose-response curve when proangiogenic molecules are measured [67] These experiments suggest that too little and too much of this drug leads to a loss of therapeutic effect. In a phase I clinical trial of endostatin, the same U-shaped dose-response curve was found when surrogate markers such as tumor blood flow and microvessel density were examined [106]. Additionally, other known endogenous angiogenesis inhibitors, such as TSP-1 and angiostatin, exhibit similar dose-dependent effects on endothelial cell and neutrophil migration *in vitro* [107-109]. However, in patients with advanced renal cell carcinoma, a bevacizumab (anti-VEGF) dose of 10 mg/kg gave significantly better results when compared to 3 mg/kg, despite prior preclinical studies showing that 3 mg/kg provides an optimal inhibition of VEGF signaling [101]. These results indicate that finding an optimal dose is not easy but yet critical for appropriate therapeutic response.

Another issue is the duration of antiangiogenic therapy, in patients that respond. In many cases, angiogenesis inhibitors will stop tumor growth, but the pre-existing tumor tissue does not disappear. Even if the endothelial cells are in fact eradicated by the treatment, the empty casts of the blood vessels still persist in the tumor matrix, and these casts can potentially function as tracks for regrowth of vessels once the drug treatment is halted [110]. Also, microscopic tumor nodules or micrometastases can persist for long periods of time, without the need for neovascularization, a process known as tumor dormancy [111]. Clinical data from long-term anti-VEGF therapy in patients with stage IV renal cell carcinoma indicate that treatment should be continued as long as the patients respond and tolerate it well [101].

An alternative strategy for angiogenesis inhibition is the use of vascular disrupting agents (VDA's). While antiangiogenic drugs arrest the growth of new vessels, VDA's destroy preformed blood vessels and therefore have a higher propensity to regress established solid neoplasms [112]. Cytotoxic drugs belonging to the class of tubulin disrupting agents, such as vinblastine and combretastatins, are potent vascular disrupting agents [112,113]. In lower doses these same drugs exhibit antiangiogenic activity and may reduce tumor blood flow by inducing endothelial edema within the tumors [15,53,54,114,115]. The combination of such agents with other treatment modalities, such as radiation or local heating of solitary tumors, can mediate increased vascular damage and tumor growth delay [54,112]. There is limited data to suggest that VDA's might augment the activity of angiogenesis inhibitors. In rhabdomyosarcoma xenografts, the effect of the angiogenesis inhibitor TNP-470 was not increased by combination with combretastatin A-4 [116]. However, in two other murine tumor models, increased tumor growth delay was observed when the angiogenesis inhibitor ZD6474 was combined with the VDA ZD6126 [117]. The potential of VDA therapy to promote improved survival in cancer patients is currently being assessed in various clinical trials [118].

Matrix Metalloproteinase Inhibitors and Their Lack of Efficacy in Clinical Trials

Endothelial cells and tumor cells use matrix metalloproteinases (MMPs) to degrade the extracellular matrix and basement membranes as the new blood vessels sprout inside a growing tumor [119,120]. Inhibitors of MMPs (MMPIs) to stop tumor angiogenesis and

cancer progression, showed great promise in preclinical tumor experiments [121-123]. However, in large phase III clinical trials MMPIs failed to show prolongation of overall survival [121,123-125]. On the contrary, there was even a tendency towards increased metastasis risk and shorter survival for cancer patients receiving the drugs [123,126].

Looking back at the preclinical studies there were already indications that MMPIs were not effective. While halting solid tumor growth in a breast cancer model, the MMPI batimastat had no effect on ascites development or tumor cell invasion [127]. In a rat glioma model, batimastat also had insignificant effect on solid tumor growth [16].

The failure of MMPIs in clinical trials was suggested to be due to a lack of efficacy in large, advanced tumor masses, as compared to inhibition of angiogenesis in small tumors in an adjuvant setting [125,128]. Also, MMPs can in fact have both proangiogenic and antiangiogenic effects, via proteolytic activation of proangiogenic cytokines or liberation of endogenous angiogenesis inhibitors like tumstatin, arresten and canstatin [124,129]. Thus, the administration of MMPIs can inhibit angiogenesis by arresting proteolytic activation of proangiogenic cytokines, or promote angiogenesis by inihibiting the liberation of endogenous angiogenesis inhibitors from the extracellular matrix [37]. In this context, integrin α1 deficient mice exhibit diminished tumor angiogenesis and reduced vascularity in the skin due to increased activity of MMP7 and MMP9 which generates angiostatin from plasminogen. So an inhibitor that inactivates MMP7 and MMP9 would reduce the level of angiostatin and thus increase angiogenesis [130]. The potential response also depends on whether one uses a broad-spectrum or a narrow-spectrum MMPI [124]. Another possible reason for MMPIs failure to arrest tumor growth, might be connected to the upregulation of other proteases, such as serine and cysteine proteases, when MMPs are inactivated [131]. Finally, lack of patient compliance is a possible factor in the failure, as some patients stopped taking the MMPI drugs due to painful musculoskeletal side effects [125].

Future Aspects

It is evident that angiogenesis inhibitors currently approved for clinical use are not providing long term efficacy. Drug resistance commonly develops after a few months of therapy, and improvements are clearly needed. The use of combinations of drugs targeting different angiogenic growth factors (“antiangiogenic cocktails”), or drugs that target multiple angiogenesis pathways, such as sorafenib and sunitinib, might exhibit better efficacy.

Another strategy is to obtain tumor biopsies to analyze growth factors that are upregulated in a particular patient, in order to administer “tailor-made” antiangiogenesis treatment. For instance phosphatase and tensin homolog (PTEN) can be used as a biomarker. Loss of PTEN renders anti-EGFR therapy ineffective [132]. One can also assess the level of VEGFR-2^+ circulating endothelial cells as a surrogate marker for the efficacy of antiangiogenic therapy [95,133]. The use of biomarkers for antiangiogenic response is a major focus of current research [134]. With the advancement of DCE-MRI and new ultrasound techniques, future clinical trials can potentially be designed so that these imaging modalities can guide the timing of chemotherapy administration related to induced changes in tumor blood perfusion.

Antiangiogenic therapy may be more potent if administered at an early stage as adjuvant therapy, after curative surgery, radiotherapy or high-dose chemotherapy. At this stage of disease, the antiangiogenic drugs may prolong patient survival substantially by keeping micrometastases in check. Currently, several clinical studies are being carried out to address these approaches [135,136]. In general, angiogenesis inhibitors are an important class of anti-cancer agents. But the current treatment response is transient, and more research is therefore required to address why drug resistance occurs.

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Abbreviations

eGFP	enhanced green fluorescent protein
TSP-1	thrombospondin-1
VEGF	vascular endothelial growth factor
VEGFR-22	VEGF receptor-2
FGF	fibroblast growth factor
SDF-1	stromal cell-derived factor-1
PDGFR-α	platelet derived growth factor receptor-α
EGFR	epidermal growth factor receptor
PI3K	phosphatidylinositol-3-kinase
RTK	receptor tyrosine kinase
ERK	extracellular signal-regulated kinase
mTOR	mammalian target of rapamycin
STAT3	signal transducer and activator of transcription protein 3
TGFβ	transforming growth factor β
Alk5	activin-like kinase 5
αSMA	α-smooth muscle actin
HIF-1α	hypoxia-inducible factor-1α
MTD	maximal tolerated dose
VDA	vascular disrupting agent
MMP	matrix metalloproteinase
MMPI	MMP inhibitor
PTEN	phosphatase and tensin homolog

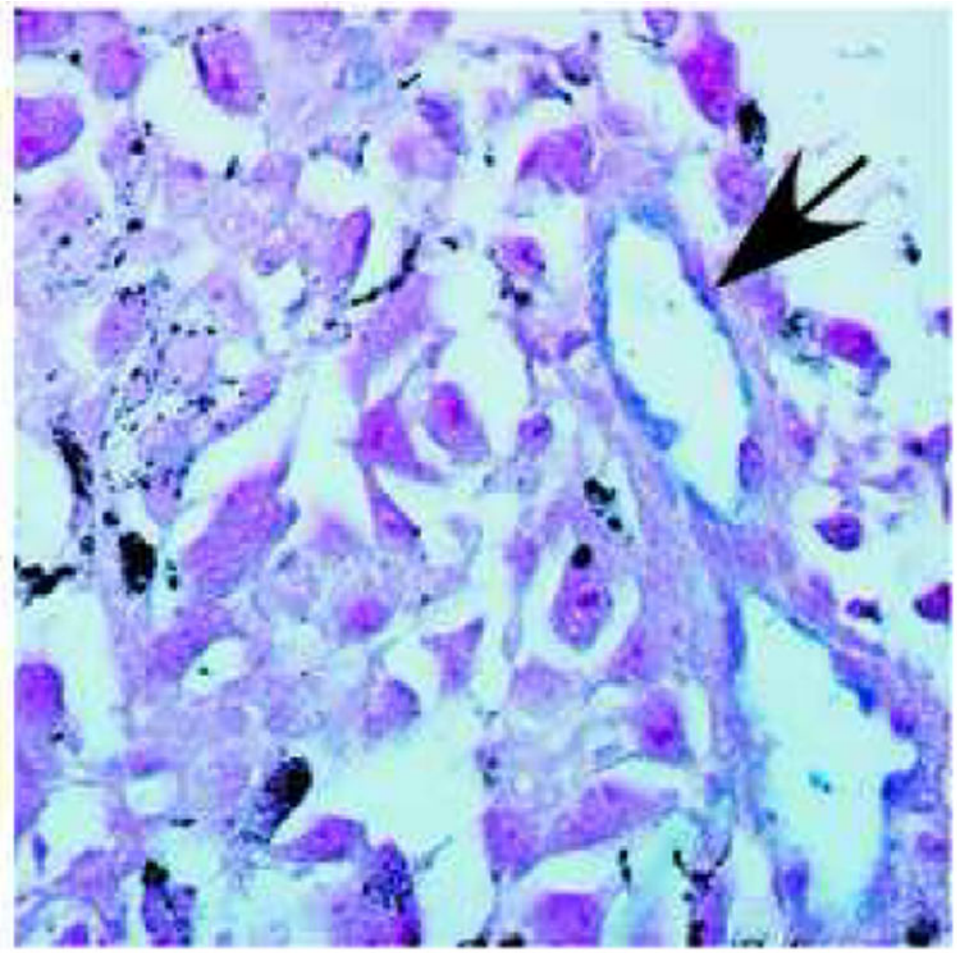

Figure 1.

LacZ staining of a LacZ$^-$ B16F10 melanoma grown in a Tie2-LacZ$^+$ mouse. All endothelial cells in the tumor vasculature are LacZ$^+$ (blue), indicating that the neovasculature is recruited from the surrounding normal tissue. Arrow pointing to a blood vessel. Red: neutral red counterstain. LacZ staining was performed as described previously [17]. 600× magnification.

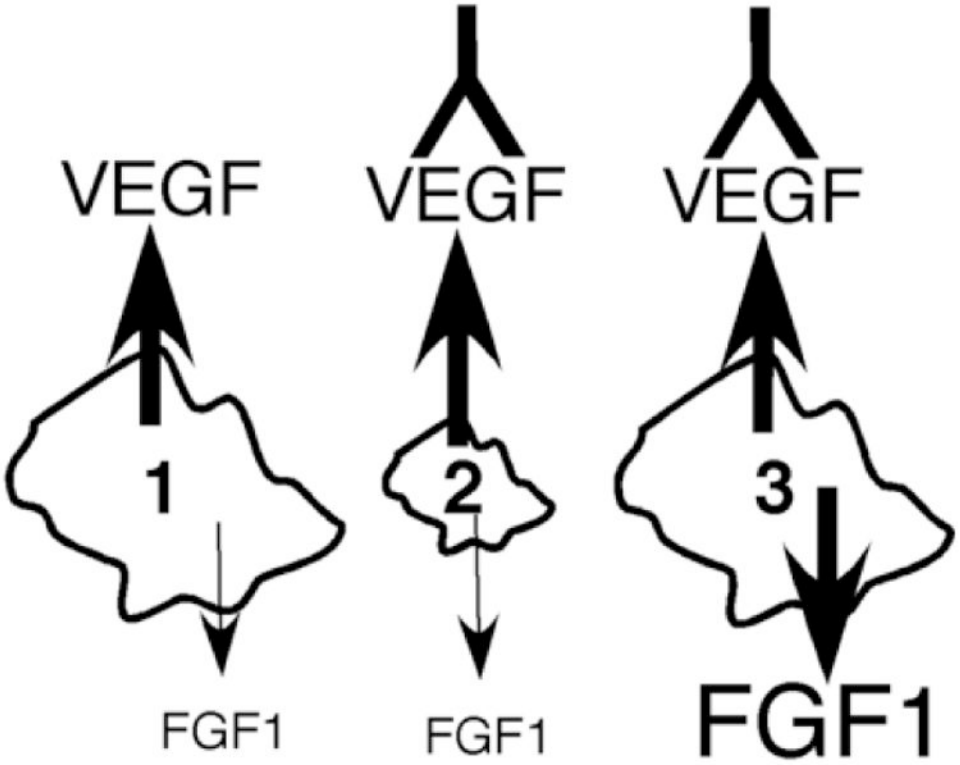

Figure 2.

Signal redundancy in a tumor upon treatment with anti-VEGF antibody. 1. Before any treatment initiated, 2. Anti-VEGF treatment regresses tumor, 3. FGF1 production is upregulated in the tumor and the rescued angiogenic response causes the tumor to regrow.

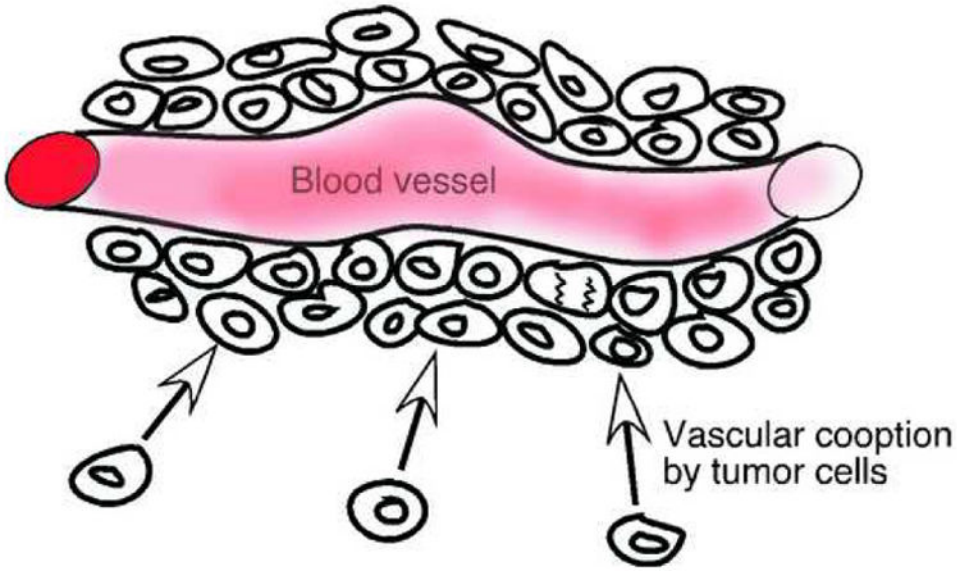

Figure 3.

Vascular cooption, a phenomenon whereby tumor cells grows around pre-existing blood vessels in the normal tissue in stead of initiating angiogenesis.

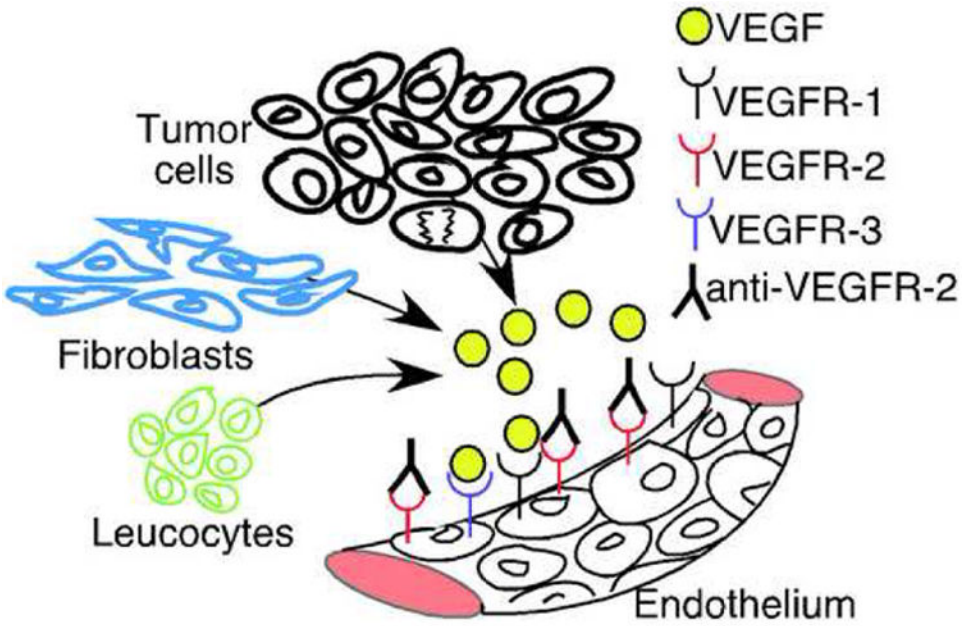

Figure 4.

Fibroblasts, leucocytes and tumor cells secrete VEGF and can stimulate angiogenesis via VEGFR-1 and VEGFR-3, despite anti-VEGFR-2 therapy.

Table 1

Endogenous angiogenesis inhibitors with direct endothelial cytotoxicity.

Angiogenesis inhibitor	Receptors and biological effect on endothelial cells	Reference
Angiostatin	Binds to angiomotin, αvβ3 integrin and other receptors and affects several signaling pathways to arrest the cell cycle and induce apoptosis.	[56-58]
Arresten	Binds to α1β1 integrin and inhibits migration and proliferation via effects on several signaling pathways.	[59,60]
Canstatin	Binds to αvβ3 and α3β1 integrin. Inhibits migration and induces apoptosis via FLIP downregulation.	[61,62]
Endorepellin	Binds to α2β1 integrin and inhibits cell migration via disassembly of actin cytoskeleton and focal adhesions.	[63,64]
Endostatin	Binds to α5β1 and other receptors and affects various signaling pathways to arrest the cell cycle and induce endothelial apoptosis. Disassembly of actin stress fibers.	[56,65-68]
Hexastatin	Not known.	[69]
Platelet factor-4	Binds to heparin-like glycosaminoglycans. Inhibits cell cycle and matrix-associated proteases.	[56]
16-kDa N-terminal fragment of prolactin	Unknown receptor. Affects several signaling pathways to arrest the cell cycle and induce apoptosis.	[56]
Thrombospondin	Binds to CD36 and αvβ3 integrin and inhibits several intracellular pathways. Inhibits matrix-associated proteases.	[56,70]
Tumstatin	Binds to αvβ3 and α6β1 integrin and inhibits protein synthesis.	[44,71]