Chapter 9
Resistance to Inhibitors of Angiogenesis

Nili Dahan, Ksenia Magidey, and Yuval Shaked

Abstract Angiogenesis, a process that is predominantly driven by the vascular endothelial growth factor (VEGF) signaling pathway, plays an essential role in tumor progression and metastasis. Accordingly, a range of anti-angiogenic agents, most of which block VEGF or its receptor, have been approved for the treatment of various malignant diseases. However, the clinical benefits of anti-angiogenic therapy are relatively modest for several reasons, some of which are related to the development of therapy resistance. Since anti-angiogenic agents target the tumor-supporting vascular system rather than the tumor cells themselves, resistance is dependent on the interplay between the host- and tumor-mediated pathways. In general, the activation of various evasive mechanisms allows for sustained tumor vascularization and growth despite the therapeutic blockade of the drug target. These mechanisms include the upregulation of bypass angiogenic pathways, pro-angiogenic activity of infiltrating stromal cells and alternative vascularization processes. In addition, off-target effects of anti-angiogenic drugs have implications for tumor aggressiveness. In this chapter, we discuss the molecular and cellular mechanisms contributing to therapy resistance as well as possible strategies to improve the clinical outcome.

Keywords Chemokines • Chemokine receptors • Tumor microenvironment • Angiogenesis • Bone marrow-derived cells

Abbreviations

BMDC	Bone marrow-derived cell
CAF	Cancer-associated fibroblast
CRC	Colorectal cancer

N. Dahan • K. Magidey • Y. Shaked (✉)
Department of Cell Biology and Cancer Science, Rappaport Faculty of Medicine, Technion—Israel Institute of Technology, 1 Efron St. Bat Galim, Haifa 31096, Israel
e-mail: yshaked@tx.technion.ac.il

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ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
GBM	Glioblastoma multiforme
G-CSF	Granulocyte colony stimulating factor
GIST	Gastrointestinal stromal tumor
HCC	Hepatocellular carcinoma
HGF	Hepatocyte growth factor
HIF-1	Hypoxia inducible factor-1
MDSC	Myeloid-derived suppressor cell
NSCLC	Non-small-cell lung cancer
OS	Overall survival
PDGF	Platelet-derived growth factor
PFS	Progression-free survival
PlGF	Placental growth factor
PNET	Pancreatic neuroendocrine tumor
RCC	Renal cell carcinoma
SCF	Stem cell factor
SDF-1α	Stromal derived factor-1α
TAM	Tumor-associated macrophage
TEM	Tie2-expressing monocyte
TH17	T helper type 17
TKI	Tyrosine kinase inhibitor
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

9.1 Introduction

Angiogenesis, the formation of new blood vessels from existing vasculature, plays an essential role in tumor progression and metastasis. The angiogenic process involves the activation, proliferation and migration of endothelial cells toward angiogenic stimuli produced by the tumor and supporting stromal cells within the tumor microenvironment. This ultimately results in the formation of new blood vessels that supply the growing tumor with nutrients and oxygen. This “angiogenic switch” is recognized as a rate-limiting event in tumor progression [1]. The concept of anti-angiogenic therapy was first proposed over four decades ago by Judah Folkman. He postulated that since the growth of all solid tumors is dependent on angiogenesis, inhibiting this process should suppress tumor growth [2]. It is now well-established that one of the most potent factors driving angiogenesis, and especially tumor angiogenesis, is the vascular endothelial growth factor-A (VEGF-A).

Based on the key role of this factor in tumor angiogenesis, numerous therapies that block various components of the VEGF signaling pathway have been developed [3]. Several such therapies have been approved for the treatment of a variety of human cancers and there are more in preclinical and clinical trials. However, despite the potent activity of these agents and the high expectations for this therapeutic strategy, the clinical benefits are proving to be relatively mild. In the majority of patients, anti-angiogenic therapy achieves transient tumor control, with only a modest gain in long-term survival [4]. This can be explained by several mechanisms of resistance that allow the tumor to evade the therapeutic inhibition of angiogenesis. Here we discuss the molecular and cellular events underlying resistance in different tumor contexts, distinguishing between tumor- and host-mediated mechanisms.

9.2 Inhibitors of Angiogenesis: Mode of Action and Clinical Use

Although angiogenesis is a highly complex process, it is driven by one predominant key player, VEGF-A (hereafter, referred to as VEGF) in both physiological and pathological conditions. VEGF signals through its main receptor expressed on endothelial cells, VEGFR2, thereby coordinating the biological processes necessary for new vessel formation. These processes include: endothelial cell proliferation, migration, invasion and survival; chemotaxis and homing of bone-marrow-derived endothelial precursor cells; vascular permeability; and vasodilation [3, 5]. Whereas autocrine VEGF, released by endothelial cells, maintains vascular homeostasis [6], paracrine VEGF, released by both tumor cells and stromal myeloid cell types, increases vessel branching resulting in abnormal, tortuous vasculature [7]. VEGF is upregulated in most solid tumors. Furthermore, slight increases in tumor VEGF levels are sufficient to promote angiogenesis and tumor growth. Accordingly, it was proposed that neutralizing circulating VEGF would suppress tumor growth, as demonstrated by a number of cancer models in mice [3, 8].

Since 2004, several drugs that target VEGF or its receptor have been approved by the Food and Drug Administration (FDA) for the treatment of various malignant diseases (Table 9.1), and there are more in clinical trials. These drugs include neutralizing antibodies against VEGF and VEGFRs, soluble VEGF receptor hybrids (VEGF traps) and tyrosine kinase inhibitors (TKIs) with selectivity for VEGFRs. It should be noted that, due to their mode of action at the ATP-binding pocket, TKIs designed to target VEGFRs may also significantly inhibit other kinases. Nevertheless, their potent anti-angiogenic activity has been demonstrated in preclinical studies [3]. Bevacizumab, a monoclonal antibody against VEGF, was the first anti-angiogenic drug to be approved by the FDA. It is currently used as first-line therapy in metastatic colorectal cancer (CRC), non-small-cell lung cancer (NSCLC) and renal cell carcinoma (RCC), as second-line therapy in CRC and glioblastoma multiforme (GBM), and as maintenance therapy in advanced ovarian cancer (Table 9.1).

Table 9.1 Clinical benefits of approved anti-angiogenic drugs

Drug	Drug class	Approved use	Treatment combination	Improvement in RR (%)	Improvement in PFS (months)	Improvement in OS (months)	Ref.
Bevacizumab	VEGF-A antibody	Metastatic CRC	Chemotherapy	10	4.4	4.7	[139]
				14.1	2.6	2.1	[140]
				0	1.4	1.4	[141]
		Metastatic NSCLC	Chemotherapy	20	1.7	2	[142]
				10.3–14	0.4–0.6	NS	[143, 144]
		Metastatic RCC	IFNα	12.4	3.3	NS	[145, 146]
				18	4.8	2	[147, 148]
		Advanced ovarian cancer	Chemotherapy	19	NS	NS/4.8[a]	[149, 150]
				NA	3.8	NS	[151]
		GBM	Monotherapy	Only phase II data reported			[152]
Ramucirumab	VEGFR2 antibody	Metastatic GEJ	Chemotherapy	12	1.5	2.2	[153]
			Monotherapy	0.8	0.8	1.4	[154]
Aflibercept	VEGF trap	Metastatic CRC	Chemotherapy	8.7	2.2	1.4	[155]
Sorafenib	TKI	Metastatic RCC	Monotherapy	8	2.7	NS	[156, 157]
		Metastatic HCC	Monotherapy	1	NS	2.8	[158]
Sunitinib	TKI	Metastatic RCC	Monotherapy	25	6	4.6	[159, 160]
		Metastatic GIST	Monotherapy	NA	20.9	NA	[161]
		PNET	Monotherapy	9.3	5.9	NA	[162]
Pazopanib	TKI	Metastatic RCC	Monotherapy	27	5	NS	[163, 164]

Drug	Drug class	Approved use	Treatment combination	Improvement in RR (%)	Improvement in PFS (months)	Improvement in OS (months)	Ref.
Axitinib	TKI	Advanced RCC	Monotherapy	NA	2.6	NS	[165][b]
Regorafenib		Metastatic CRC	Monotherapy	0.6	0.2	1.4	[166]
Nintedanib	TKI	Advanced NSCLC	Chemotherapy	NA	0.7	1	[167][c]
Vandetanib	TKI	Advanced medullary thyroid cancer	Monotherapy	43	6.2	NA	[168]
Cabozantinib	TKI	Advanced medullary thyroid cancer	Monotherapy	28	7.2	NS	[169]

CRC colorectal cancer, *GBM* glioblastoma multiforme, *GEJ* gastric and gastroesophageal junction cancer, *GIST* gastrointestinal stromal cancer, *HCC* hepatocellular carcinoma, *NA* not available, *NS* not significant, *NSCLC* non-small-cell lung cancer, *OS* overall survival, *PFS* progression-free survival, *PNET* pancreatic neuroendocrine tumors, *RCC* renal cell carcinoma, *RR* response rate, *TKI* tyrosine kinase inhibitor

[a]In poor-prognosis patients

[b]Axitinib vs. sorafenib

[c]Approved only in Europe

Of note, bevacizumab generally failed to provide significant benefits when used as monotherapy. However, with the exception of GBM, it has been approved for use as combination therapy for the treatment of the above-mentioned advanced-stage cancers [9]. The TKIs, sorafenib, sunitinib, pazopanib and axitinib are approved as monotherapies for the treatment of metastatic RCC, a highly vascularized tumor type. In addition, sunitinib is approved for gastrointestinal stromal tumors (GIST) and pancreatic neuroendocrine tumors (PNET), and sorafenib for hepatocellular carcinoma (HCC), for advanced-stage disease in all cases (Table 9.1). Other anti-angiogenic therapies approved for late-stage, metastatic disease are described in Table 9.1. These include: ramucirumab, a VEGFR2 monoclonal antibody; aflibercept, a VEGF-trap that binds 3 VEGF family ligands; and other VEGFR TKIs. Anti-angiogenic agents have also been evaluated for early-stage disease, specifically in the adjuvant setting, when treatment is administered after surgical removal of the primary tumor. It has been postulated that inhibiting angiogenesis after tumor resection would prevent local relapse or growth of micrometastases [10]. However, two large phase III post-operative adjuvant trials of bevacizumab in combination with chemotherapy in patients with early-stage CRC failed to provide significant benefits when compared to treatment with chemotherapy alone [11–13]. The use of anti-angiogenic therapy in the neo-adjuvant setting in order to downsize or downstage a tumor before resection has also been evaluated. However, two large trials testing the efficacy of neoadjuvant bevacizumab in combination with chemotherapy in comparison to neoadjuvant chemotherapy alone in patients with primary breast cancer revealed conflicting findings in terms of long-term benefits [14, 15]. The diverse outcomes of anti-angiogenic therapy in different clinical scenarios highlight the effects of specific parameters, such as disease stage and cancer type, on therapy efficacy. However, our understanding of the underlying mechanisms is still incomplete.

In general, protein-based anti-angiogenic drugs, such as bevacizumab and aflibercept, have only shown significant activity when combined with cytotoxic chemotherapy, whereas TKIs are effective when used as monotherapy, without an additive effect when combined with chemotherapy [10]. Conceivably, in cases where single-agent activity is observed, such as in RCC, therapy-induced vessel regression is the major mechanism of action contributing to the efficacy of therapy. In cases where anti-angiogenic therapies only show activity when combined with cytotoxic chemotherapy, such as in CRC, mechanisms other than vessel regression may play a role [3]. A widely-held view is that anti-angiogenic therapy improves the delivery of co-administered chemotherapy through a process known as “vascular normalization”. This is based on the principle that the abnormal tumor vasculature, which is known to be dysfunctional, leaky and tortuous, can be “normalized” by suppressing VEGF signaling. The resulting improvement in vessel function and blood flow is presumed to increase delivery of cytotoxic agents [16]. An alternative possibility explaining the benefit of combined therapy is that anti-angiogenic agents block the activity of bone marrow-derived endothelial progenitor cells that have been shown

to infiltrate tumors in response to chemotherapy drugs [8, 17–19]. However, given that the clinical relevance of such phenomena is dependent on cancer type and drug class, additional unknown mechanisms likely play a role [4]. A recent study suggests that the vessel phenotype of tumors contributes to the response to different treatment strategies. Using preclinical models and clinical samples, it was shown that cancers that are more responsive to bevacizumab in combination with chemotherapy have a stromal-vessel phenotype, where the vessels are surrounded by a well-developed stroma. In contrast, cancers that are more responsive to TKI monotherapy have a tumor-vessel phenotype, where the vessels are in close proximity to the tumor cells [20]. In addition, tumor-specific differences likely account for why certain anti-angiogenic therapies show efficacy in some cancers, but not in others, although the precise molecular mechanisms are not known [10].

Although anti-angiogenic therapy has been incorporated into the standard protocol for certain cancer types, there are a number of concerns, the foremost being its modest clinical benefits. The gain in progression-free survival (PFS) and overall survival (OS) is generally in the order of months. In addition, initial response rates and gains in PFS do not always translate into significant improvements in OS (Table 9.1). These limited clinical benefits strongly suggest that tumors treated with anti-angiogenic agents develop resistance to therapy. Such resistance can be classified as intrinsic, where tumors are unresponsive from the beginning of treatment, and acquired, where tumors initially respond but then progress during the course of treatment [21]. Thus, there is an urgent need to overcome these limitations and to develop improved strategies for the treatment of cancer at all stages of the disease.

9.3 Mechanisms of Resistance to Inhibitors of Angiogenesis

There is a growing interest in understanding the mechanisms underlying both acquired and intrinsic resistance to anti-angiogenic therapy. Classical drug resistance mechanisms involve the clonal selection of tumor cells harboring genomic mutations that either alter the drug target or affect drug uptake or efflux [22]. However, since anti-angiogenic therapy targets the vascular supply of the tumor mass rather than the tumor cells themselves, resistance in this case is mainly indirect and involves an interplay between tumoral cues and host-mediated pathways. In addition, given that endothelial cells are more genetically stable than tumor cells, they are less likely to acquire mutations after exposure to such drugs [23]. In general, resistance to anti-angiogenic therapy is manifested by the activation of alternative mechanisms that sustain tumor vascularization and growth while the specific target of the drug remains inhibited [21]. These evasive mechanisms are described in detail below. A graphical summary is shown in Fig. 9.1.

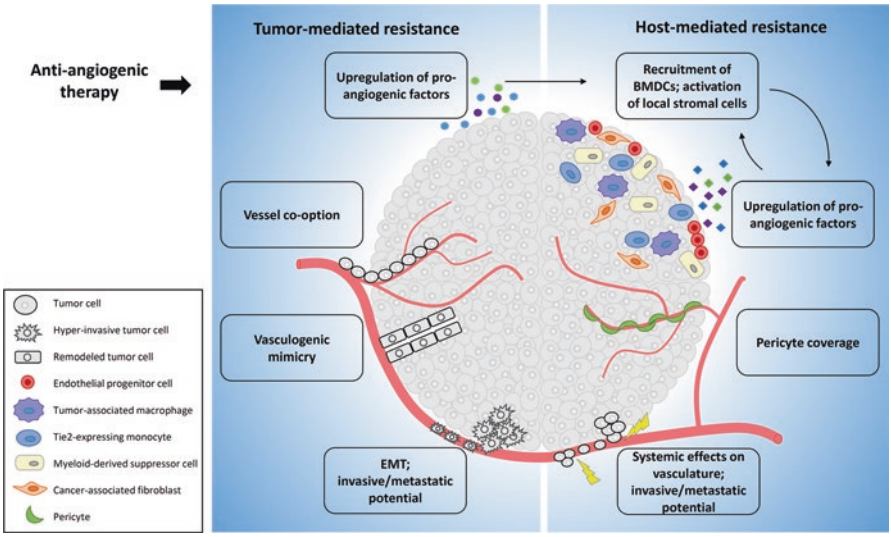

Fig. 9.1 Mechanisms of resistance to anti-angiogenic therapy. Tumors develop resistance to anti-angiogenic therapy via a range of tumor- and host-mediated processes. These evasive mechanisms sustain tumor vascularization and/or progression despite the blockade of VEGF signaling imposed by anti-angiogenic agents. Increased tumor hypoxia, which occurs as a direct result of anti-angiogenic therapy, drives many of these processes. The processes are not mutually exclusive, and some are interdependent (indicated by thin arrows). BMDC, bone marrow-derived cell; EMT, epithelial-mesenchymal transition

9.3.1 Upregulation of Alternative Angiogenic Pathways

Tumor angiogenesis is mainly driven by the VEGF signaling pathway. However, there are also numerous complementary non-VEGF pathways that contribute to blood vessel formation. Tumor hypoxia, which occurs as a direct result of anti-angiogenic therapy, modulates the interplay between these various angiogenic pathways via the master regulator, hypoxia inducible factor-1 (HIF-1), a transcription factor that regulates the expression of multiple pro-angiogenic genes [24]. The activation of alternative or compensatory angiogenic pathways allows for persistent neovascularization despite VEGF inhibition and represents the most common means by which tumors evade the blockade of angiogenesis. Preclinical trials in a murine pancreatic cancer model demonstrated an initial response to anti-VEGFR2 therapy (DC101) followed by restoration of tumor growth and vascularization shortly after initiation of therapy. Interestingly, at the time of progression, these tumors expressed higher levels of various pro-angiogenic factors such as fibroblast growth factor (FGF) 1 and 2, ephrin A1 and A2 and angiopoietin 1. Similarly, tumor cells subjected to hypoxic conditions upregulated most of these genes. Blocking both VEGF and FGF signaling attenuated revascularization and slowed tumor growth, suggesting that upregulation of FGF signaling contributes to anti-angiogenic therapy resistance [25]. Several additional pro-angiogenic factors have been implicated in

resistance to anti-angiogenic therapy in various murine tumor models. These include: placental growth factor, PlGF [26]; platelet-derived growth factor, PDGF [27]; hepatocyte growth factor, HGF, and its receptor, c-Met [28, 29]; epidermal growth factor, EGF [30]; interleukin-8, IL-8 [31]; granulocyte colony stimulating factor, G-CSF, and Bv8 [32], among others (recently reviewed in [33]). Similar to the seminal study of Casanovas et al. [25], the above-mentioned studies report elevated levels of the specific factor in resistant tumors and that dual inhibition of the VEGF pathway and the specific factor or its pathway enhances therapeutic outcome. It should be noted that these upregulated pro-angiogenic factors may be derived from tumor cells or host stromal cells residing within the tumor microenvironment. The former case involves a direct response of tumor cells to hypoxia. In the latter case, stromal cells may be responding to cues from the tumor, environmental signals or systemic effects of the drug [10, 34].

There is a wealth of clinical evidence showing that circulating levels of pro-angiogenic factors are elevated just prior to disease progression or during the relapse phase in cancer patients treated with angiogenesis inhibitors suggesting that these factors contribute to the development of acquired resistance [35–40]. There are also cases in which patients do not respond at all to anti-angiogenic therapy suggestive of intrinsic resistance. In late stage malignancies, pre-existing upregulation of alternative pro-angiogenic pathways may compensate for the inhibition of VEGF signaling [21].

9.3.2 Pro-angiogenic Effects of Local and Bone Marrow-Derived Stromal Cells

The release of pro-angiogenic factors in response to anti-angiogenic therapy activates local stromal cells and stimulates the recruitment of bone marrow-derived cells (BMDCs) to the tumor environment. BMDCs include vascular progenitors, which differentiate into cells that make up physical components of the blood vessel walls, and pro-inflammatory monocytes, which produce a diverse assortment of soluble factors that regulate vascular cell survival, proliferation and motility as well as extracellular matrix (ECM) remodeling [21, 41].

The effect of hypoxia on BMDC recruitment was described by Du et al. in an orthotopic model of GBM. They demonstrated that HIF-1α, the direct effector of hypoxia, promotes angiogenesis and tumor growth by inducing an influx of various pro-angiogenic bone-marrow derived $CD45^+$ myeloid cells as well as endothelial and pericyte progenitor cells to the tumor [42]. In addition, treating tumor-bearing mice with vascular-disrupting agents, which cause massive tumor hypoxia, triggers an acute mobilization of circulating endothelial progenitor cells that home to the tumor margins in sufficient numbers to facilitate revascularization [17]. Thus, therapy-induced hypoxia represents a major contributing factor to resistance via the action of recruited BMDCs.

The involvement of specific BMDC types and local stromal cells in resistance to anti-angiogenic therapy is described below:

Immature myeloid cells, also known as $CD11b^{+}Gr1^{+}$myeloid-derived suppressor cells (MDSCs), produce a variety of factors that influence endothelial cell behavior resulting in new vessel formation [41]. Shojaei et al. demonstrated that tumors resistant to anti-VEGF therapy exhibit higher levels of infiltrating MDSCs in comparison to therapy-sensitive tumors [43]. This is due to an upregulation of G-CSF and Bv8 [32], factors that promote the mobilization of MDSCs from the bone marrow and their infiltration to tumor tissue [44]. In pancreatic tumor models that are resistant to anti-VEGF therapy, increased levels of proinflammatory factors including several CXCR2 ligands, IL-1α, IL-1β and Angptl-2 stimulate the recruitment of $CD11b^{+}$ myeloid cells to the tumor environment [45]. In agreement with the above-mentioned studies, blocking chemotherapy-induced infiltration of MDSCs to tumors using Bv8 neutralizing antibodies enhances therapy outcome in mouse models of pancreatic cancer [46]. In another study, it was suggested that tumor-infiltrating T helper type 17 (TH17) cells and IL-17 induce the recruitment of immature myeloid cells to the tumor microenvironment. Blocking TH17 cell function renders resistant tumors sensitive to anti-VEGF therapy [47].

Tumor-associated macrophages (TAMs) are recruited to tumors as monocytes from the circulation and, as they extravasate across the tumor vasculature, they differentiate into macrophages. In the tumor environment, TAMs are predominantly polarized towards an M2-like phenotype underlying their ability to promote tumor growth and angiogenesis [48]. TAMs promote angiogenesis mostly through their production of VEGF [41]. However, TAM-derived PlGF can also stimulate angiogenesis in some tumors, representing a possible mechanism for acquired resistance to VEGF/VEGFR-targeted therapies [49]. In HCC xenografts, sorafenib increases CXCL12 levels and TAM infiltration. Furthermore, depletion of TAMs enhances the inhibitory effect of therapy on tumor angiogenesis, growth and metastasis demonstrating the contribution of TAMs to therapy resistance [50].

Tie2-expressing monocytes (TEMs) represent a distinct subpopulation of TAMs expressing the angiopoietin receptor, Tie2. They physically associate with vessels and secrete growth factors and matrix-remodeling proteins that stimulate the angiogenic process in a paracrine manner [51]. TEMs are recruited and activated via endothelial cell- and tumor-secreted chemoattractants, Ang2 and CXCL12, respectively [52, 53]. Their recruitment to spontaneously growing tumors promotes angiogenesis [54]. Furthermore, TEMs infiltrate hypoxic tumors treated with a vascular-disrupting agent, and inhibiting such infiltration enhances treatment efficacy [53]. Lastly, dual targeting of VEGF and Ang2 has been shown to delay tumor growth and improve the outcome of anti-angiogenic therapy in preclinical studies [55–57]. These collective findings highlight the possible contribution of TEMs to resistance to anti-angiogenic therapy.

Pericytes, the periendothelial support cells of the microvasculature, are derived from local or bone marrow-derived mesenchymal stem cells. They provide important support for blood vessel formation, structure and function. Furthermore, tight cross-talk between pericytes and endothelial cells maintains blood vessel integrity

[58]. While anti-angiogenic therapy reduces tumor vascularity, the vessels that remain are functional, distinctively thin and tightly covered with pericytes [21, 59, 60]. Owing to their important role in maintaining vessel integrity, the remaining pericytes, along with basement membrane-associated cells, facilitate a rapid regrowth of blood vessels after cessation of treatment with angiogenesis inhibitors [61]. Importantly, pericytes mediate endothelial cell quiescence and survival and therefore their presence presumably reduces responsiveness to anti-angiogenic therapy [21]. The underlying molecular mechanism involves pericyte-induced survival signals that induce an autocrine activation loop of VEGF signaling and anti-apoptotic Bcl-w expression in tumor endothelial cells [62]. Additional pericyte-derived endothelial survival signals, specifically via the Ang1/Tie2 and EGF pathways, may also contribute to anti-angiogenic therapy resistance [30, 63]. Accordingly, it has been suggested that targeting both endothelial cells and pericytes may improve the efficacy of anti-angiogenic therapy. Indeed, such dual targeting improves therapy outcome in a variety of murine tumor models [63–65]. However, severe reduction in pericyte coverage damages the integrity of the vasculature, enabling local intravasation of tumor cells thereby facilitating metastasis [66]. In support of this concept, a recent study demonstrated that TKI-induced pericyte depletion enhances metastasis due to increased vessel leakiness and hypoxia-associated epithelial-mesenchymal transition (EMT) [67]. Collectively, enhanced as well as reduced pericyte coverage contribute to anti-angiogenic therapy resistance via different mechanisms.

Cancer-associated fibroblasts (CAFs) are tumor-localized, activated fibroblasts originating from connective tissue fibroblasts proximal to neoplasms or from local and bone marrow-derived mesenchymal stem/progenitor cells. They promote angiogenesis by producing a variety of pro-angiogenic signaling factors, chemoattractants and ECM-degrading enzymes [41]. Crawford et al. showed that the upregulation of PDGF-C in CAFs from anti-VEGF resistant tumors compensates for the inhibition of VEGF-dependent angiogenesis. Furthermore, CAFs isolated from resistant tumors can stimulate the growth of therapy-sensitive tumors even when VEGF is inhibited. This suggests that, once activated by the tumor environment, CAFs retain their ability to induce angiogenesis independent of tumor cells [27].

9.3.3 Alternative Vascularization Mechanisms

Primary tumors and metastases may gain access to a blood supply via mechanisms that are independent of classical sprouting angiogenesis. These alternative vascularization mechanisms are not affected by antiangiogenic drugs and therefore represent another mode of resistance to such therapy [68].

Vessel co-option refers to the migration of tumor cells along existing and established blood vessels in the host organ to invade healthy tissue. This process is mostly observed in highly vascularized tissues such as brain, lungs and liver, where tumor cells can co-opt the abundant pre-existing blood vessels [69]. Preclinical and clinical

data show that glioblastomas become more infiltrative with the use of anti-angiogenic therapy, facilitating vessel co-option [42, 70–74]. In addition, vessel co-option has been implicated in resistance to anti-angiogenic therapy in HCC [75] and metastases in lymph nodes [76], brain [77], liver [78] and lung [79].

Vasculogenic mimicry is a mechanism by which highly aggressive tumor cells form vessel-like structures in an angiogenesis-independent manner. These vessel-like structures may connect to the endothelial-lined vasculature to provide a perfusion pathway for the transport of fluid, nutrients and oxygen to the core of the malignant mass [68, 80]. Since its first description in uveal melanoma [81], vasculogenic mimicry has been observed in several tumor types and is associated with poor prognosis [82]. By virtue of their plasticity, tumor cells can dedifferentiate and acquire expression of vascular markers thereby "mimicking" endothelial cells during this process [81, 83, 84]. However, despite expression of various vascular markers, such tumor cells are resilient to treatment with angiogenesis inhibitors [84–87]. Furthermore, antiangiogenic treatment has been shown to induce vasculogenic mimicry in preclinical models of various cancers [86, 87]. This may be due to treatment-induced hypoxia that upregulates vasculogenic mimicry pathways in tumor cells [88, 89]. Collectively, anti-angiogenic therapy not only triggers alternative vascularization mechanisms, but may also select for more aggressive tumor cells with an intrinsic ability to evade the blockade of angiogenesis.

9.3.4 The Host Response to Inhibitors of Angiogenesis: Implications for Tumor Aggressiveness

Targeting the host-mediated angiogenic process that supports tumor growth has its limitations. As detailed in the previous sections, anti-angiogenic therapies may trigger an array of evasive mechanisms that involve the activity of host cells such as pro-inflammatory myeloid cells and endothelial progenitor cells in the tumor microenvironment. Furthermore, anti-angiogenic therapy has been shown to augment the invasive and metastatic potential of tumors despite overall inhibition of tumor growth [90, 91]. This seemingly paradoxical phenomenon is proposed to arise, at least in part, from a direct response of the host to anti-angiogenic therapy, independent of the tumor. Ebos et al. showed that short-term sunitinib treatment of mice prior to intravenous injection of tumor cells accelerates metastasis and reduces survival. Similarly, adjuvant short-term sunitinib treatment after resection of the primary tumor enhances spontaneous metastatic tumor burden [90]. The mechanisms underlying this effect may involve a drug-induced change in the levels of circulating factors implicated in tumor progression. For example, healthy, tumor-free mice treated with VEGF receptor TKIs exhibit a dose-dependent increase in the levels of circulating G-CSF, SDF-1α, SCF and osteopontin demonstrating a systemic tumor-independent response to therapy [92]. Similarly, cancer patients treated with sunitinib exhibit increased circulating levels of

pro-angiogenic factors [93, 94]. In theory, such systemic host-mediated responses could promote the formation of "pre-metastatic niches" in distant organs, thereby facilitating metastasis [95]. The deleterious effect of anti-angiogenic therapy on the host vasculature represents another factor that may explain increased metastasis in response to such therapy. The systemic action of VEGF receptor TKIs may damage the integrity of the vasculature by targeting endothelial cells as well as pericytes. This facilitates local intravasation of invasive tumor cells and creates permissive niches for extravasation of tumor cells in target organs [67, 96, 97].

It should be emphasized that several steps are required for disease progression from a local primary tumor to established metastatic disease. These include loss of cellular adhesion, increased motility, intravasation, survival in the bloodstream, homing, extravasation, seeding of micrometastases, and finally colonization and growth at a distant site [98]. Therefore, it is conceivable that the above-mentioned host-mediated responses act in concert with tumor-derived effects to promote overall tumor aggressiveness in response to anti-angiogenic therapy. Paez-Ribes et al. demonstrated that the anti-VEGFR2 antibody, DC101, and VEGF receptor TKI, sunitinib, promote local primary tumor invasion and metastasis in mouse models of pancreatic neuroendocrine carcinoma and glioblastoma. The researchers suggest that therapy-induced hypoxia in the primary tumor triggers a switch to a hyperinvasive condition in tumor cells [91]. In agreement with this, several preclinical studies demonstrate that VEGF-targeted therapies cause tumor cells to undergo hypoxia-associated EMT, thereby promoting invasion and metastasis [67, 99, 100]. Collectively, both host- and tumor-dependent responses to anti-angiogenic therapy contribute to the invasive and metastatic potential of treated tumors.

Whether anti-angiogenic therapy causes increased tumor aggressiveness in patients is still a debatable issue. A retrospective analysis found no evidence for accelerated tumor growth in metastatic RCC patients treated with sunitinib [101]. Similarly, a meta-analysis of several randomized phase III trials of bevacizumab found no evidence for accelerated disease progression after discontinuation of therapy in patients with metastatic renal, pancreatic, breast and colorectal cancer [102]. On the other hand, rapid tumor regrowth has been reported after treatment discontinuation in RCC patients receiving sunitinib or sorafenib [103, 104], and in CRC patients receiving bevacizumab and chemotherapy [105]. In addition, several clinical studies describe an increased infiltrative growth pattern of glioblastomas in response to anti-angiogenic therapy [70, 71, 73]. The differences in preclinical and clinical findings may be explained by the animal model used, tumor type, disease stage, drug type, dosage, duration of treatment, or combination with chemotherapy [10].

Other anti-cancer treatment modalities, such as chemotherapy, radiation and surgery, can also produce undesirable pro-angiogenic and pro-metastatic effects that arise from the response of the host to therapy. Accordingly, blunting this host response using combinatorial therapies may improve treatment outcomes [34]. For example, the elevation in circulating endothelial progenitor cell levels following treatment with chemotherapeutic or vascular-disrupting agents can be blocked using anti-VEGF or anti-VEGFR2 neutralizing antibodies. This combinatorial treatment

enhances therapy efficacy and delays tumor regrowth in comparison to cytotoxic therapy alone [18]. Recent preclinical studies suggest that the reverse may be true as well; cytotoxic therapy can be used to blunt tumor aggressiveness induced by anti-angiogenic drugs thereby improving treatment efficacy. For example, concurrent paclitaxel chemotherapy was shown to block the increase in primary tumor local invasion and distant metastases induced by anti-VEGFR2 antibody (DC101) therapy in mouse models of breast cancer [106]. In addition, co-administration of chemotherapy counteracted the sunitinib-induced increase in metastasis in mice bearing early stage Lewis lung carcinoma [107]. Thus, add-on therapy that counteracts host- or tumor-dependent responses represents a possible strategy to overcome increased tumor aggressiveness and resistance in response to anti-angiogenic therapy.

9.4 Future Directions

The limited clinical benefits of anti-angiogenic therapy contrast with findings of preclinical studies conducted over the last two decades that demonstrate treatment efficacy. This can be explained by the disparity between preclinical models used to test efficacy and clinical scenarios. Due in part to ethical issues, patients enrolled in clinical trials are generally at an advanced stage of the disease. In contrast, preclinical experimental setups mostly involve localized primary tumors, with suppression of tumor growth after a short-term drug exposure considered a sign of efficacy. Therefore, more relevant preclinical models should be used to study the effects of anti-angiogenic therapy at all stages of disease, including metastatic and adjuvant settings, with clinically-relevant endpoints [108].

In theory, alternative pro-angiogenic pathways upregulated in response to anti-angiogenic therapy may be targeted as a strategy to overcome resistance. Multi-targeted inhibitors such as brivanib, a dual VEGFR and FGFR TKI, and nintedanib, a triple angiokinase inhibitor for VEGFR, FGFR and PDGFR, are being tested in clinical trials [109, 110]. Importantly, host-mediated evasive mechanisms induced in response to anti-angiogenic therapy may also be targeted in order to improve anti-angiogenic therapy outcomes. The major BMDC recruiting factor, SDF1α (CXCL12), represents a potential target for cancer therapy. Recent preclinical and clinical data support the use of anti-CXCL12 agents to reduce BMDC infiltration as a potential strategy to overcome resistance to anti-angiogenic therapy [18, 111]. Macrophages are key regulators in the tumor microenvironment, and have been implicated in resistance to anti-angiogenic therapy. Therefore, specifically blocking macrophage infiltration is also a potential means for overcoming resistance. Antibodies against the monocyte chemotactic protein, CCL2, and the macrophage-expressed CSF-1 receptor are being tested in clinical trials as monotherapies [112–114]. It will be interesting to test whether such agents synergistically increase efficacy when combined with anti-angiogenic agents in the clinical setting [115].

As detailed throughout this review, hypoxia resulting from anti-angiogenic therapy drives tumor aggressiveness and therapy resistance via tumor- and host-mediated mechanisms. Therefore, alleviating hypoxia or targeting HIF-1 represent avenues for future investigation [116]. The former case would involve optimizing the dosage and scheduling of anti-angiogenic agents with the aim of normalizing the abnormal tumor vasculature as opposed to inducing rapid and excessive vessel pruning [16]. Indeed, tumor perfusion and oxygenation correlates with clinical benefit in GBM patients treated with anti-angiogenic therapy [117–120]. Alleviating hypoxia would reduce processes such as EMT, vasculogenic mimicry and the selection of more aggressive tumor cells as well as affect immune and stromal cells within the tumor microenvironment. It is well-established that a hypoxic tumor environment induces BMDC recruitment and reprograms TAMs towards a pro-tumorigenic phenotype. Therefore, alleviating hypoxia through vascular normalization could potentially reprogram the entire tumor microenvironment [16]. Histone deacetylase inhibitors have been shown to strongly repress HIF-1 expression and their use as anti-cancer drugs is currently being explored [121]. A recent phase I clinical trial evaluating the use of a histone deacetylation inhibitor in combination with the anti-angiogenic agent, pazopanib, demonstrated durable tumor regression in 70% of patients with pazopanib-refractory disease [122]. Thus, epigenetic targeting represents a potential strategy to reverse resistance to anti-angiogenic therapy, possibly by targeting HIF-1. The precise molecular mechanisms and clinical benefits should be further characterized.

The combination of anti-angiogenic drugs with immunotherapy represents an emerging strategy for cancer treatment. The rationale for using this combination is based on the systemic influence of VEGF on immune cell function. Specifically, several studies have demonstrated that an elevated level of circulating VEGF in tumor-bearing hosts impedes immune surveillance and destruction of tumor cells [123–125]. Accordingly, anti-angiogenic drugs may be used to neutralize the immunosuppressive activity of VEGF. Moreover, the combination of anti-angiogenic therapy with immunotherapy could potentially offer a synergistic anti-cancer effect. In addition, it has been proposed that alleviating tumor hypoxia via vascular normalization would reprogram the phenotype of the tumor microenvironment from immunosuppressive to immunosupportive, thereby improving the efficacy of anti-cancer immunotherapies [16, 126]. A number of preclinical and clinical studies have demonstrated the benefits of this combination strategy [127–133].

Lastly, a major challenge is to identify robust biomarkers predictive of clinical efficacy of anti-angiogenic therapy. Currently, no validated biomarkers exist to select patients who will benefit from such therapy. Biomarkers under consideration in various cancers include circulating VEGF-A, VEGF-D, Ang2, HGF, osteopontin, IL6 and IL8, among others [4, 134]. With respect to VEGF as a predictive biomarker for bevacizumab-based treatment benefit, phase III trials have reported a correlation between high circulating levels of VEGF and survival benefit in metastatic breast and gastric cancer patients [135, 136], but not in CRC, RCC and lung cancer patients [137]. Other emerging areas for biomarker identification include tumor vessel imaging with dynamic contrast-enhanced MRI, measurement of circulating endothelial

cells, expression arrays, single nucleotide polymorphisms and early pharmacodynamic response to treatment, such as hypertension [138]. The incorporation of predictive biomarkers into routine clinical practice would maximize clinical benefit, reduce unnecessary toxicity and improve costs of cancer care.

9.5 Conclusions

The development of anti-angiogenic agents is an important milestone in the field of cancer research. However, their clinical use is proving to be more complex than originally anticipated with major ongoing challenges. A prominent issue in the clinic is resistance to therapy resulting in only modest gains in long-term survival in the majority of patients. Given that anti-angiogenic agents target the tumor-supporting vascular system comprised of a variety of host cells, and that tumor progression is regulated by tumor-host cell cross-talk, resistance is dependent on both tumor- and host-mediated mechanisms (Fig. 9.1). Understanding these mechanisms is key to developing strategies to overcome therapy resistance and improve clinical outcome.

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