Resistance to Angiogenesis Inhibitors in Renal Cell Carcinoma

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Abstract: Antiangiogenic drugs are now available for treatment of renal cell carcinoma and are utilized sequentially to prolong clinical benefit in patients with recurrent disease. These antiangiogenic agents are disease stabilizing in most cases, and resistance eventually develops over time. Because different combinations and sequences are tested in clinical trials, resistance patterns and mechanisms should be investigated. Much effort has been devoted to understanding the biology and elucidating the pathways and additional targets during tumorigenesis and metastasis. Resistance appears to be either primary nonresponsiveness, or it is acquired over time and related to various evasive/escape mechanisms that the tumor develops in response to therapy. Primary resistance is less common, but may be due to an intrinsic redundancy of available angiogenic signals for the tumor, causing unresponsiveness to vascular endothelial growth factor (VEGF)-targeted therapies. During acquired resistance, tumors may activate an "angiogenic switch," which leads to either upregulation of the existing VEGF pathway or recruitment of alternative factors responsible for tumor revascularization. Rationally designed preclinical and clinical trials will shed additional light on our understanding of the potential mechanisms of resistance to antiangiogenic drugs.

Keywords
Antiangiogenesis, resistance, renal cell carcinoma, VEGF

Introduction

Renal cell carcinoma (RCC) comprises approximately 2% of all malignancies, with a median age at diagnosis of 65 years. More than 54,000 new cases of carcinoma of the kidney and renal pelvis were estimated for 2010, with approximately 13,000 deaths.[1] It is characterized by variable clinical presentations, ranging from a very indolent course to an aggressive clinical progression and unusual sites of metastasis.[2]

The administration of high-dose bolus interleukin-2 has historically produced consistent, durable responses in a small percentage of patients with advanced RCC,[3] but because of its toxicity, its

use is limited to select centers across the United States. With the advent of targeted therapy, the paradigm for the treatment of RCC has significantly changed. January 2006 saw the approval of sunitinib (Sutent, Pfizer) for the treatment of advanced kidney cancer by the US Food and Drug Administration (FDA) in quick succession after sorafenib (Nexavar, Bayer) was approved in December 2005. The FDA subsequently has also approved temsirolimus (Torisel, Wyeth), everolimus (Afinitor, Novartis; for patients who do not respond to receptor tyrosine kinase inhibitors [TKIs]), bevacizumab (Avastin, Genentech) in combination with interferon alpha (IFN-α), and more recently pazopanib (Votrient, GlaxoSmithKline).[4]

With the slew of targeted agents now available, several agents have been tested in various combinations and sequences to prolong progression-free survival and extend overall survival. With the advent of targeted therapies, the median survival of patients with metastatic RCC has increased from approximately 12 months in the IFN-α era up to 40 months.[5] Different agents using alternate pathways can be used with some benefit. Also, rechallenge with the same agent has been found to be potentially useful.[6] However, despite recent success, patients do progress through vascular endothelial growth factor (VEGF)-targeted therapies.

Drug resistance in the context of antiangiogenic inhibitor use still needs to be clearly defined. Response Evaluation Criteria In Solid Tumors (RECIST) are utilized to evaluate responses to therapy during a clinical trial. However, the decision to either continue or change course of therapy is often based on clinical judgment rather than strictly radiologic progression. It is recognized that the criteria used to define disease response need to be readdressed because the pattern of progression is different when compared to nontargeted and traditional chemotherapies. Studies are being conducted to evaluate other criteria and imaging methods, such as the contrast-enhanced computed tomography (CT).[7] There is no consensus as yet on how to more adequately define the criteria for drug resistance to targeted therapy in RCC. Often, responses with the use of targeted agents are characterized by change in appearance of lesions without size change, thus defying the traditional RECIST-based assessment of progression. An accepted criterion for progression is the development of new lesions in the absence of progression in existing tumor volume. Potentially, resistance could present as primary or intrinsic (preexisting) nonresponsiveness, where a small subset of patients is refractory to therapy at the time of initial response assessment.[8] A larger group of patients manifests tumor regression early in the course of therapy. This regression is followed by a plateau in tumor burden over a short interval, and then disease progression several months from the start of treatment. A substantial subgroup of patients has tumor regression in the first several months of therapy followed by a prolonged plateau in tumor size and absence of new lesions.[9]

A recent concept is that success in cancer treatment can be defined as converting cancer to a chronic disease with long-term stability. The goal of therapy is still largely viewed as "achieving some degree of tumor shrinkage" for as long as possible, with optimum quality of life.[10] Hence, the clinical benefit of drugs that inhibit the VEGF pathway is limited, since they fail to produce enduring and/or complete clinical responses in the majority of patients.

Genetics, Biology, and Molecular Targets

Von Hipple-Lindau and Hypoxia-inducible Factor

The pathogenesis of clear cell RCC has been elucidated by the discovery of the von Hippel-Lindau (VHL) gene from the study of VHL syndrome families. *VHL* is a tumor suppressor gene, located on the short arm of chromosome 3 (3p25-26).[11] Tumors arise from the biallelic *VHL* gene inactivation. One of the alleles is inactivated through a deletion, as observed in more than 90% of noninherited (sporadic) clear cell RCC.[12] The remaining *VHL* allele is inactivated either through gene mutation, seen approximately in 50% of clear cell RCC,[13,14] or through gene silencing by methylation in 5–10% of cases.[15,16] However, alterations at other loci, other than the VHL loci, are probably required for carcinogenesis to take place.[11,17] *VHL* encodes the VHL protein, which is a component of an E3 ubiquitin-ligase complex that targets a transcription factor, hypoxia-inducible factor (HIF-α), for proteasome-mediated degradation. Inactivation of VHL leads to the formation of a defective VHL protein and HIF is not degraded, leading to its accumulation. Several lines of evidence suggest that HIF-α, and in particular HIF-2α, plays a causal role in clear cell carcinoma. Through transcriptional regulation, HIF enhances glucose uptake and increases expression of VEGF, platelet-derived growth factor β polypeptide (PDGFβ), plasminogen activator inhibitor-1 (PAI-1), erythropoietin, and transforming growth factor α (TGFα).[18]

Hypervascular neoplasms, such as RCC, are dependent on overproduction of these growth factors, which bind to specific receptors and activate receptor tyrosine kinases (RTKs).[13] HIF-induced growth factors are mainly involved in tumor angiogenesis by stimulation of the endothelial cell compartment. Activation of the RTK VEGF receptors (VEGFRs) results in endothelial cell proliferation, migration, invasion, and survival. Activation of the PDGF receptor-βs (PDGFR-βs) may provide

mechanical support to vasculature through pericytes, which are able to protect endothelial cells from apoptosis in the setting of VEGFR blockade.[14]

VEGF

On the basis of significant upregulation of VEGF in RCC derived from preclinical models, small-molecule inhibitors of the VEGFR, PDGFR, and related receptors, including sunitinib and sorafenib, are thought to exert their major therapeutic effect in RCC by antagonism of VEGFR, leading to reduced tumor angiogenesis.[19-22] Although the mechanism of *VHL* inactivation and resulting VEGF overexpression appears to be clear, many studies have failed to show a direct association between either *VHL* status or VEGF levels and clinical response to VEGF-targeting agents.[23]

Mammalian Target of Rapamycin (mTOR)

Another effective target of therapy in RCC is the mTOR pathway. Studies have shown the effectiveness of targeting this pathway similarly to the VEGF pathway. Regulation of mTOR pathway activation is complex. mTOR integrates a variety of signals that reflect cellular growth stimuli, nutrient availability, energy status, and stress. Biochemical studies placed mTOR in the growth factor–activated phosphatidylinositol 3-kinases (PI3K) Akt (protein kinase B) signaling pathway, downstream from Akt.[24] The mTOR pathway functions through 2 multiprotein complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2).[25] TORC2 is implicated in the control of cell morphology and adhesion. TORC2 has also been shown to phosphorylate and activate Akt.[26] Through phosphorylation of 2 downstream effectors, p70S6 kinase and the binding protein for eukaryotic initiation factor 4E, TORC1 controls the translation of cyclin D, c-Myc, and other key proteins involved in cell proliferation. TORC1 also regulates the expression and stability of HIF-1α.[27,28] Thus, mechanisms underlying the antitumor activity of temsirolimus and everolimus in RCC probably include inhibition of both angiogenesis and tumor cell proliferation. Importantly, mTOR kinases associated with TORC2 may be relatively resistant to complete inhibition by rapamycin in vitro in some cell lines, raising the question whether the activity of TORC2 may be a potential mechanism of resistance.[29] Loss of VHL function in RCC also results in deregulation of cyclin D1, a cyclin-dependent kinase cofactor required for cell cycle progression.[30,31]

Histone Deacetylase (HDACs)

HDACs are enzymes that regulate the status of chromatin. HDACs remove the acetyl group from lysine residues of the tails of histone proteins, whereas histone acetyltransferases (HATs) attach the acetyl group to the tails of the histone proteins. Increased acetylation of histones leads to open chromatin and increased transcription, whereas deacetylation of these histones leads to closed chromatin and transcriptional repression. A common finding in cancer cells is a high level expression of HDAC isoenzymes and a corresponding hypoacetylation of histones.[32,33] In the presence of oncogenic transformation, HIF-1α protein is known to be stabilized. Genetic and epigenetic silencing of VHL induces the overexpression of HIF-1α, which is translocated into the nucleus to regulate gene expressions such as the VEGF. HDAC inhibitors may induce a degradation of HIF-1α and hence limit the formation of VEGF.[34] The HDAC inhibitor SNDX 275 (Syndax Pharmaceuticals) has demonstrated antitumor activity in a preclinical model of human RCC.[34] Although there is evidence of HDAC overexpression in RCC and other cancers, it is not clear whether the overexpression correlates with or is predictive of response to HDAC inhibitors.[35]

In a recent study, our group tested the safety and efficacy of the HDAC inhibitor vorinostat (Zolinza, Merck) and the VEGF inhibitor bevacizumab (Avastin, Genentech) in patients previously treated with VEGFR TKIs.[36] Patients with stage IV clear cell RCC with up to 2 prior regimens were eligible. Treatment consisted of vorinostat 200 mg orally twice daily for 2 weeks, and bevacizumab 15 mg/kg intravenously every 3 weeks. We observed 6 objective responses (18%), including 1 complete response (prior sunitinib) and 5 partial responses. Nineteen patients (67%) had stable disease (12–84+ weeks). To date, the median progression-free survival and overall survival are 5.3 months and 16.2 months, respectively. These preliminary results suggest that the combination of vorinostat with bevacizumab is well tolerated, has clinical activity in previously treated RCC, and represents a rational strategy to overcome resistance to VEGFR TKIs that should be tested in future clinical trials.

Mechanisms of Resistance

Drug resistance is acquired by mutational alteration of the gene encoding a drug target or by alterations in drug uptake and efflux.[37,38] Resistance to antiangiogenic agents is not likely to be secondary to a mutational alteration of a gene. Compared to genetically unstable tumor cells, the endothelial cells that are recruited by tumors to form the tumor vasculature are proposed to be genetically more stable,[39-41] and resistance to TKIs in the tumor endothelium has not been reported.

Bergers and Hanahan have proposed 2 general models of resistance to angiogenesis inhibitors, in particular

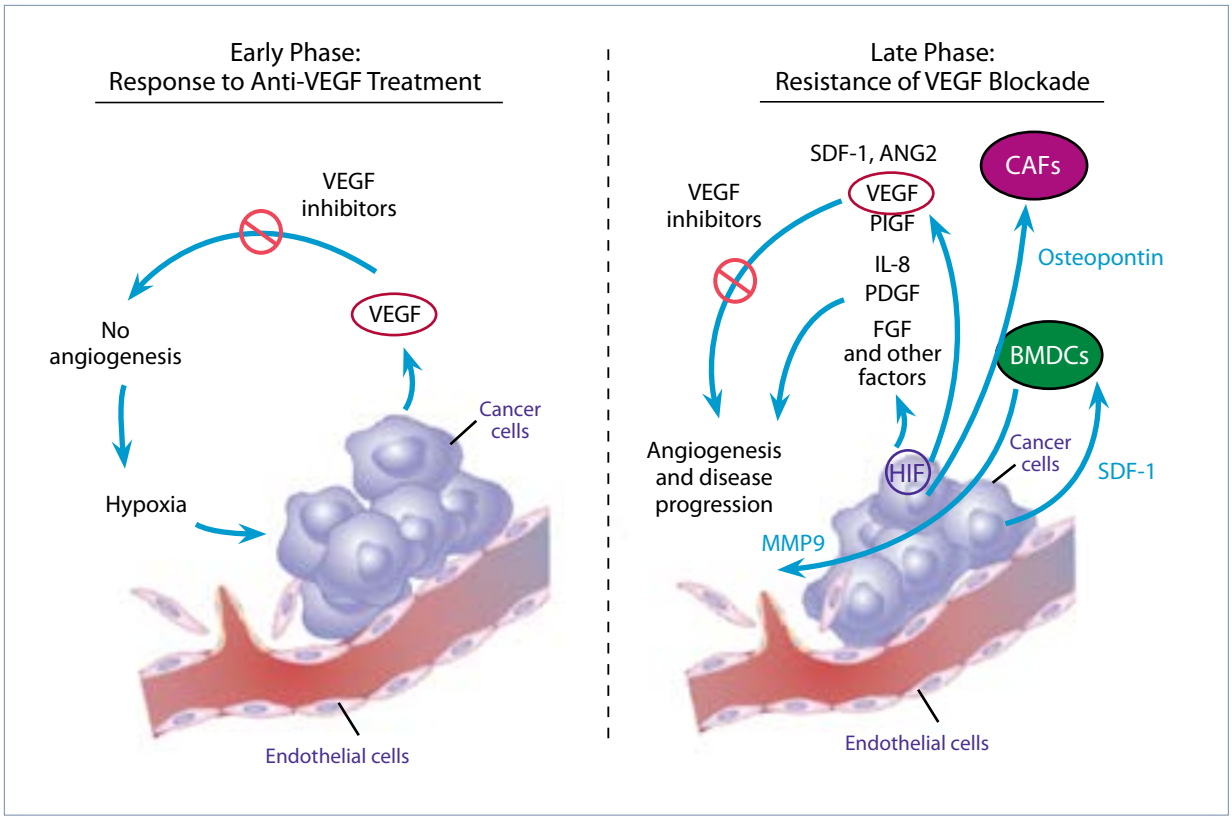

Figure 1. Hypoxia-inducible factor (HIF) and resistance to anti–vascular endothelial growth factor therapy (VEGF).

ANG=angiopoietin; BMDC=bone marrow–derived cells (ie, myeloid-derived suppressor cells); CAF=cancer-associated fibroblasts; FGF=fibroblast growth factor; MMP9=matrix metallopeptidase 9; PDGF=platelet-derived growth factor; PlGF=placental growth factor; SDF=stromal cell–derived factor

Adapted from Casanovas O, et al. *Cancer Cell.* 2005;8:299-309.

those targeting the VEGF pathways: adaptive or evasive resistance, and intrinsic or primary resistance.[42] The evolving hypothesis is that angiogenic tumors adapt to the presence of angiogenesis inhibitors and acquire the means to functionally evade the therapeutic blockade of new blood vessel formation.[41,43] The "angiogenic switch" is recognized as a rate-limiting event in multistage carcinogenesis, as documented in animal cancer models, and is correlated in advanced premalignant stages, as well as in their malignant progression. Tumors may activate this angiogenic switch by changing the balance of angiogenesis inducers and countervailing inhibitors.[44] The driving force of this angiogenic switch may be represented by HIF induced by the hypoxic conditions as consequences of the initial anti-VEGF therapies (Figure 1).[45-47]

A common strategy for shifting the balance may involve altered gene transcription. Many tumors demonstrate increased expression of VEGF and/or fibroblast growth factors (FGFs) compared to their normal tissue counterparts. In others, expression of endogenous inhibitors such as thrombospondin-1 or IFN-β is downregulated. This observation suggests that mechanisms of an angiogenic or a VEGF escape take place. These mechanisms may include upregulation of alternative proangiogenic signaling pathways by development of alternative circuits for angiogenesis, upregulation of the hypoxia responsive angiogenic growth factors such as VEGF, and modulation of the angiogenic signals by tumor stromal compartment.[48] Upregulation of existing pathways and proangiogenic proteins may also play a role, which may lead to inadequate target inhibition. There is no conclusive clinical evidence yet to support dose escalation as a method to overcome this resistance, though some reports suggest a dose-dependent effect of response and/or lack of response to VEGF TKIs.[49]

Primary Resistance

It is conceivable that certain tumors are intrinsically resistant to antiangiogenic therapy. In this case, tumors fail

to show even a transitory clinical response. Disease continues to progress with no signs of shrinkage or stability. In some patients the primary resistance could be relative. The VEGF pathway may still be activated, but the degree of suppression by the selective drugs may not be adequate. Pharmacokinetic variability and VEGF receptor polymorphisms could be responsible.

In most of the cases, primary resistance may be related to the existing plethora of angiogenic signals. For example, there is a redundancy in the downstream intracellular signaling of VEGF or bFGF, and a specific single network component may be more involved than others. Hence, inhibition of a single component may not be adequate to slow tumor growth. A preclinical study demonstrated that a subset of murine transplant tumors growing in mice showed no responsiveness to an anti-mouse VEGF monoclonal antibody.[50] This study reveals CD11b+Gr1+ monocytes to be mediators of intrinsic resistance to anti-VEGF treatment in murine transplant tumors. The nonresponsive tumors, which had not previously been treated with chemotherapy, were characterized by a pre-existing infiltration of inflammatory cells, principally CD11b+Gr1+ myeloid cells, which were shown to express a number of proangiogenic factors. By contrast, the responsive tumor types had comparatively low levels of such inflammatory cells. Pharmacologic impairment of myeloid cell recruitment rendered the otherwise resistant tumors responsive to the VEGF blockade. Even though this may not provide conclusive evidence, additional studies in this direction may provide clues for the mechanisms responsible for intrinsic resistance.

Antiangiogenic Therapy Evasion

There is some evidence for the existence of evasive resistance manifested by alternative proangiogenic signaling. A genetically engineered mouse model (Rip1–Tag2) of pancreatic neuroendocrine (islet cell) cancer treated with a monoclonal antibody (DC101) blocking VEGFR2 initially responded as tumor stasis with attenuation of tumor vascularity. This response was transitory (10–14 days), followed by tumor regrowth, suggesting that a permanent genetic or epigenetic change in the tumor cells was unlikely. There was evidence of restoration of dense tumor vasculature indicative of reinitiation and persistence of tumor angiogenesis. The relapsing tumors were found to express higher levels of the mRNAs for the proangiogenic factors fibroblast growth factor 1 (FGF1) and FGF2, ephrin A1 and A2, and angiopoietin (Ang)-1. These tumors had regions of acute hypoxia at the peak of the response phase, and tumor-derived cells subjected to hypoxic conditions similarly upregulated most of these genes. In order to assess the functional significance of these upregulated genes, mice were first treated with the VEGFR inhibitor alone, and then at the peak of the response they were treated with an FGF trap (FGFR-Fc fusion protein) to suppress signaling through the FGF ligands. The combination attenuated the revascularization and slowed tumor growth, indicating that FGF signaling was involved in regulating the restored angiogenesis.

Interleukin-8

Interleukin-8 (IL-8) has been shown to have a dominant role in the generation and maintenance of tumor microcirculation. In a study involving HIF-1-α knockdown in colon cancer cell lines, VEGF expression was preserved and a neutralizing anti-IL-8 antibody blocked tumor angiogenesis.[51] In another tumor model, escape to sunitinib coincided with increased secretion of IL-8 from tumors into the plasma, and coadministration of an IL-8 neutralizing antibody resensitized tumors to sunitinib treatment.[52] In patients who were refractory to sunitinib treatment, tumor IL-8 expression was elevated, supporting the concept that IL-8 levels might predict clinical response to sunitinib. IL-8 may be an important contributor to sunitinib resistance in RCC, and a candidate as a therapeutic target to reverse acquired or intrinsic resistance to sunitinib in this malignancy.

Placental Growth Factor

Hypoxia appears to be the driving force for the development of these alternate pathways and proteins as inducers of tumor angiogenesis. Similarly, placental growth factor (PlGF), a VEGF homologue, has been implicated as a potential alternate pathway to growth of VEGFR inhibitor–resistant tumors.[53] The authors attributed the inability of αVEGFR-2 to inhibit macrophage infiltration as a contributing factor to the tumor's resistance to αVEGFR-2 and, thus, propose that αPlGF treatment could be particularly valuable when combined with VEGFR-2 blockade. PlGF is a member of the VEGF family, which was initially cloned in 1991. Loss of PlGF was demonstrated to impair pathologic angiogenesis in adults, including new blood vessel formation associated with tumors.[54] Murine PlGF amplifies VEGF signaling in endothelial cells through VEGFR-1 transphosphorylation of VEGFR-2, and recombinant PlGF treatment stimulates revascularization of ischemic tissues.[55,56] PlGF levels are known to increase in the circulation of cancer patients receiving anti-VEGF treatment.[52,57,58] Fischer and colleagues[53] demonstrated that the murine PlGF-2 antibody was effective in tumor growth inhibition. Plasma levels of both VEGF and PlGF have been shown to be increased after treatment with either bevacizumab[59] in patients with rectal cancer, or with receptor TKIs in patients with recurrent glioblastoma or metastatic RCC, although a direct

correlation between VEGF levels and clinical response has not been established.[57,60] Hence, it can be conceptualized that therapies targeting PlGF may represent a rational strategy to delay resistance to anti-VEGF therapies.

It is a widely accepted notion that endothelial cells (ECs) maintain a high clonogenic potential throughout adulthood, and new blood vessels originate from the preexisting vasculature through the proliferation of endothelial cells (ECs) during angiogenesis.[61] Besides the origin of tumor ECs from preexisting vessels, it has been proposed that endothelial progenitor cells (EPCs) exist in the adult bone marrow (BM), circulate in the peripheral blood (PB), and may incorporate into new blood vessels in addition to the pericyte progenitors that develop into pericytes and embrace the blood vessels.[62-66] These cells may represent an escape mechanism for the tumor vasculature. Potentially low oxygen conditions caused by vessel regression during the course of antiangiogenic therapy may lead to the recruitment of these bone marrow–derived cells. Influx of these bone marrow–derived cells may depend on a threshold of or correlation with the degree of low oxygen tension.[67] These findings support the hypothesis that inhibition of vascular supply by angiogenic inhibitors induces tumor hypoxia, leading to "hypoxia insensitive" tumors with enhanced local invasiveness and metastatic activity. In addition, vascular modulators, such as tumor-associated macrophages, myeloid-derived suppressor cells, cancer-associated fibroblasts, and immature monocytic cells (including TIE2+ monocytes), may yield their influence by expressing a variety of cytokines, growth factors, and proteases (Figure 1).[68]

Angiopoietins

The angiopoietin/TIE system acts as a vascular-specific ligand/receptor system to control endothelial cell survival and vascular maturation. The angiopoietin family includes 4 ligands and 2 corresponding tyrosine kinase receptors (TIE1 and TIE2). Ang-1 and Ang-2 are specific ligands of TIE2, binding the receptor with similar affinity. TIE2 activation promotes vessel assembly and maturation by mediating survival signals for endothelial cells and regulating the recruitment of mural cells. Ang-1 acts in a paracrine agonistic manner inducing TIE2 phosphorylation and subsequent vessel stabilization. In contrast, Ang-2 is produced by endothelial cells and acts as an autocrine antagonist of Ang-1-mediated TIE2 activation. Ang-2 thereby primes the vascular endothelium to exogenous cytokines and induces vascular destabilization at higher concentrations. Ang-2 is strongly expressed in the vasculature of several tumors and may act synergistically with other cytokines, such as VEGF, to promote tumor-associated angiogenesis and tumor progression. Ang-2 is only weakly expressed in endothelial cells under physiologic conditions. However, Ang-2 expression is dramatically increased during vascular remodeling (eg, during tumor growth). The Ang-2/TIE2 axis appears to have angiogenic potential that could parallel the VEGF axis. Also, Ang-1- and TIE2-deficient mice appear to show severe defects in the recruitment of pericytes and in their interaction with endothelial cells.[69-71] Ang-2 inhibition has been shown to lead to the suppression of tumor growth and to stimulate the production of matrix metalloproteinases (MMPs). The consequent stimulation of VEGF secretion and Ang-2 may be responsible for denuding the endothelium, mediating vascular leak syndrome, and priming the endothelium to respond to other angiogenic factors.

PDGFR

Some models have suggested that inhibition of *PDGFR* might enhance antiangiogenic effects of TKIs by the targeting pericytes, which are able to protect endothelial cells from apoptosis in the setting of VEGFR blockade. Inhibition of VEGF signaling can lead to substantial reduction in tumor vascularity, and distinctive functional vessels that are tightly covered with pericytes continue to exist, in contrast to less closely associated pericytes in the typically dilated tumor vessels of untreated specimens.[72-76] It has therefore been suggested that endothelial cells can induce pericyte recruitment to protect themselves from death resulting from the lack of the crucial tumor-derived survival signals conveyed by VEGF. Studies have reported that tumor vessels that lack adequate pericyte coverage are more vulnerable to VEGF inhibition,[72,77] and that tumor pericytes express significant levels of VEGF and potentially other factors that support endothelial cell survival required for the proper maturation and stabilization of newly formed vascular structures.[66,78,79] Furthermore, pericytes may be capable of attenuating the proliferation rate of endothelial cells.[79,80] Hence, it is possible that pericytes mediate endothelial cell survival in treated tumors, making endothelial cells less responsive to antiangiogenic agents. Dual targeting of endothelial cells and pericytes may provide improved efficacy. This observation has been made in mouse models, including the Rip1-Tag2 transgenic mouse model of pancreatic neuroendocrine cancer using VEGF and PDGF inhibitor signaling, which targets endothelial cells and pericytes, respectively.[81,82] The absence of pericyte coverage in tumor vasculature has been found to be associated with metastasis and poorer prognosis in patients with colorectal cancer.[83] In clear cell RCC, it has been demonstrated that the undifferentiated microvessels, which are correlated with poor prognosis, are not covered by pericytes.[84] This suggests that pericyte coverage might play an important role in tumor progression in RCC as well, and resistance (either

primary or evasive) may be related to the adaptation of pericytes around the vasculature.

Epithelial-to-Mesenchymal Transition

There are some intriguing data from our laboratory suggesting that the tumor microenvironment contributes to the acquired resistance to sunitinib.[85] We have recently reported the de novo onset of an epithelial-mesenchymal-transition (EMT)-like phenotype in a patient with conventional clear cell RCC on sunitinib treatment. The patient acquired resistance to sunitinib, with the development of new skin lesions. We observed the reversion to an epithelial histology in a primary xenograft model, which was again responsive to the treatment with sunitinib. This observation raises the hypothesis that EMT and the changes in the related microenvironment may contribute to the development of resistance. EMT has been extensively studied in recent years and is the ultimate result of protein modification and transcriptional events in response to a defined set of extracellular stimuli leading to long-term, although sometimes reversible, cellular changes. Its presence has been observed in prostate cancer as well and it may be associated with aggressive prostate cancer cells that lose their luminal epithelial phenotype, including androgen receptor expression, during tumor progression.[86] Also, EMT seems to be associated with metastasis, drug resistance, and angiogenesis.[87-91] Our observation also suggests that reversal of resistance after a drug holiday—a clinical phenomenon that has been anecdotally observed—may be possible.[6] Whether EMT should be considered a potential resistance mechanism to antiangiogenic therapies in RCC awaits further studies.

Increased Tumor Cell Invasion

In addition to the above-described mechanisms for escape or evasion of the targeted circuits, there is a hypothesis that tumors adapt to antiangiogenic therapies by developing a more invasive and malignant phenotype. In glioblastoma multiforme (GBM) models, tumor burden was significantly reduced, as the exponential angiogenesis-dependent tumor growth was blocked but the disseminated invasive tumor cells survived therapy.[92] Additional evidence is provided by the Rip1–Tag2 pancreatic islet tumor model, in which the reduction in tumor vascularity evoked by the angiogenesis inhibitors was accompanied by clear signs of increased tumor cell invasiveness. Tumors could adapt by changing their patterns of spread and metastasis and by being no longer solely dependent on angiogenesis, a concept validated by change in pattern of disease progression observed in GBM. Untreated GBM often invades normal brain tissue by infiltrating as single cells into the brain parenchyma and migrating along basement membranes of ventricles, leptomeninges, and blood vessels.[92] By contrast, when GBMs were genetically or pharmacologically impaired in their angiogenic capability, tumor cells were observed to predominantly migrate as multicellular layers along normal blood vessels.

Future Directions

Therapy for RCC has made long strides in recent years. Antiangiogenic therapies are effective in RCC, but complete responses are rare. Studies to determine the most effective ways to combine these therapies and to overcome potential resistance are ongoing but may be hampered by excessive toxicities. Several questions arise as we treat these patients: Are we effectively inhibiting the selected target? Is the target the same in every patient? Is the target endothelial cell–, stromal cell– or tumor cell–specific in every tumor and metastasis? New clinical tools to determine whether a patient will respond to a certain targeting agent or whether the inhibition of an alternative target will lead to a greater clinical benefit are urgently needed.

Anti-VEGF therapy "escape" or "evasive resistance" may be approached with a therapeutic strategy aimed to achieve a "vertical" inhibition of the VEGF pathway.[93] Several current and future clinical trials are testing these strategies. For example, inhibition of tumor cell adaptation to hypoxia induced by TKIs may be achieved with HDAC or mTOR inhibitors that block the HIF pathway. These hypotheses can be tested in rationally designed clinical trials. For a disease that responds initially to a TKI but eventually progresses, a rational intervention at the time of progression could involve treating patients with an antibody that neutralizes potentially elevated levels of VEGF ("sequential monotherapy approach"). Another therapeutic strategy to delay the occurrence of anti-VEGF therapy escape is the "combination approach" (eg, by adding an HIF inhibitor or an anti-VEGF ligand antibody at the time of starting the TKI). The goal with this approach is to delay the occurrence of "evasive" resistance characterized by increased HIF dependency. A combination targeting alternative growth factors, such as FGF, PlGF, and Ang-2, is a rational strategy (Table 1). However, increased toxicity has been and will remain a major hurdle in developing combination strategies. A third potential strategy is to add or switch to a drug with a different mechanism of action (eg, from an anti-VEGF therapy to an mTOR inhibitor) at the time of disease progression without discontinuing the previous treatment. The approach of "adding" rather than "switching" would allow a continuous inhibition of a specific pathway (ie, VEGF or mTOR). As observed in prostate cancer patients, the androgen receptor in castration-resistant tumor cells may undergo mutational changes, and the aggressive tumor may now be driven by other cofactors

Table 1. Therapeutic Strategies to Overcome Resistance to Antiangiogenic Agents

Type of Resistance	Pathways Involved	Therapeutic Strategies
Adaptive or evasive resistance	Hypoxia-inducible factor	Mammalian target of rapamycin inhibitors Histone deacetylase inhibitors Antisense hypoxia-inducible factor
	Fibroblast growth factor 1	Fibroblast growth factor (FGF)-trap, anti-FGF receptor antibody, FGF receptor kinase inhibitor
	Interleukin-8	Anti-interleukin-8 antibody
	Placental growth factor (PlGF)	Anti-PlGF monoclonal antibody
	Angiopoietin/TIE system	Anti-angiopoietin 1/2 antibody TIE-2 receptor kinase inhibitors
	Pericyte coverage	Platelet-derived growth factor receptor kinase inhibitors
	Epithelial-to-mesenchymal transition	Drug holiday
Primary resistance	Redundant angiogenic factors	Combination of multireceptor kinase inhibitors and neutralizing antibodies

and pathways, though the primary driving force still needs to be continually suppressed. Again, overlapping toxicities may remain a limiting factor in developing these strategies.

In conclusion, further clinical development of angiogenesis inhibitors for RCC is needed, with an increased effort to better understand the potential mechanisms of resistance. Molecular typing of tumors in prospective clinical trials will hopefully address some of the questions related to both primary and acquired resistance. Eventually, the identification of reliable biomarkers will help distinguish patients not only with a higher chance of response to antiangiogenics, but also those who may develop early resistance. A bedside-to-bench approach will be critical to advance the field and to improve the survival benefit in recurrent RCC patients in this era of targeted, individualized therapy.

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