Targeting von Hippel-Lindau Pathway in Renal Cell Carcinoma

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Abstract
Inheritance of a defective copy of the von Hippel-Lindau (VHL) gene leads to the most common cause of inherited renal cell carcinoma (RCC). In addition, most patients with sporadic RCC have aberrant VHL. In the absence of VHL, hypoxia-inducible factor α accumulates, leading to production of several growth factors, including vascular endothelial growth factor and platelet-derived growth factor. We review here the biology of RCC and how a combination of proximal and distal block of VHL/hypoxia-inducible factor α pathway by novel targeted agents, including sunitinib, sorafenib, bevacizumab, everolimus, and temsirolimus, has led to significant improvements in progression-free survival.

Background
Renal cell carcinoma (RCC) affects ~40,000 Americans each year, resulting in >12,000 deaths in the United States and >100,000 deaths worldwide annually, making RCC one of the most lethal urologic cancers. Metastatic RCC is refractory to conventional chemotherapy (1). Until recently, over decades of drug testing, only interleukin-2 and IFN-α showed activity in RCC, with a 15% response rate (2). Thus, until recently, the standard first-line agent for metastatic RCC has been treatment with IFN or interleukin-2. Recent advances in understanding the biology and genetics of RCC have led to several novel targeted approaches, with unprecedented response rates.

RCC arises from a complex series of mutation and selection events in cells located within proximal tubules of nephrons, culminating in cancer cells that acquire characteristics allowing immortalization, evasion of apoptosis, growth in a low-oxygen environment, resistance to immunosurveillance, recruitment of angiogenic factors, invasion of the basement membrane, and ultimately distant spread (3, 4). Identification of sentinel mutations driving carcinogenesis and malignant transformation is crucial in developing targeted therapeutic strategies. An early event during the evolution of clear cell RCC is loss of function of the von Hippel-Lindau (VHL) gene, located on chromosome 3p and encoding a 213-amino acid protein (5). Inheritance of a defective copy of the VHL gene leads to VHL disease and is the most common cause for inherited clear cell RCC (for review, see ref. 6). In addition, up to 75% of patients with sporadic clear cell RCC have aberrant VHL (e.g., chromosome 3p deletion, suppressed expression, or loss-of-function base substitutions). Thus, loss of VHL function is an important sentinel event during RCC pathogenesis.

The VHL gene functions in several pathways linked with carcinogenesis, most notably the hypoxia-inducible pathway. VHL is a component of an E3 ubiquitin-protein ligase complex composed of VHL, elongin B, elongin C, and ring-box 1 (6). During normal oxygen tension, this VHL complex binds with and polyubiquinates (marking for degradation within the proteasome) the transcriptional factor hypoxia-inducible factor (HIF)-1α, HIF-2α, and HIF-3α (7, 8). Under normoxia, HIF-1α is enzymatically hydroxylated at one of two proline residues located in the oxygen-dependent degradation domain. X-ray crystallography studies with VHL complexed with HIF-1α confirmed that this hydroxylation allows for hydrogen bond-mediated complex formation between the two proteins (9). During hypoxic stress, HIFα is not hydroxylated and thus cannot ligand with the VHL complex and consequently escapes ubiquitin-mediated proteolysis. Similarly, when there is loss of VHL function, HIFα accumulates within cells and subsequently binds with its constitutively present partner HIFβ (see Fig. 1). This HIFα/β complex translocates to the nucleus, binds to HIF-responsive element, and facilitates transcription of genes, including vascular endothelial growth factor (VEGF)-A (leading to angiogenesis; ref. 10), epidermal growth factor receptor type 1 (leading to cell growth), platelet-derived growth factor (PDGF) B chain, glucose transporters (e.g., Glut1), transforming growth factor-α...
(TGF-α; ligand for epidermal growth factor receptor type 1), and erythropoietin (11); in addition, HIF increases expression of three transcriptional repressors (TCF3, ZFHX1, and ZFHX1b) of E-cadherin, thus favoring metastasis. The major consequence of loss-of-function VHL is continuous activation of HIFα, resulting in accumulation of HIF effectors and culminating in increased angiogenesis, cell growth, survival in low oxygen, better adaptation to low-pH and low-nutrient environments, and ultimately metastasis (i.e., properties are acquired during tumor evolution; refs. 3, 4). HIFα is elevated in many human malignancies, further underscoring its common significance in oncogenesis.

A major role of the HIF pathway is to up-regulate transcription of mitogenic growth factors (VEGF-A, PDGFβ, and TGF-α), which following secretion into the extracellular space and can either (a) via paracrine action, bind to RTKs located on stromal or endothelial cells, leading to stromal proliferation and angiogenesis or (b) via autocrine action, bind to RTKs located on tumor cells, leading to proliferation and survival. For example, RTKs can stimulate the mitogenic RAS/Raf/MEK pathway, as the phosphotyrosines of RTKs facilitate docking of Grb2-SOS complex, ultimately resulting in activation of Ras. The activated Ras binds to Raf-1; afterwards, Raf-1 is activated via a complex series of phosphorylation and dephosphorylation steps. Similarly, mTOR is stimulated by a phosphorylation cascade, which involves proteins, including phosphatidylinositol 3-kinase (PI3K) and AKT. Once stimulated, mTOR controls protein translation of elements involved in cell cycle progression; in addition, mTOR also controls protein synthesis of HIF-1α in RCC cells. The signal pathways in RCC can be inhibited at several steps, including the following: (a) inhibition of VEGF (by bevacizumab); (b) inhibition of tyrosine kinase activity of RTK (by sunitinib and sorafenib); and (c) inhibition of mTOR (by temsirolimus and everolimus).
Clinical Translational Advances

Because of its central role in the pathogenesis of RCC and other malignancies, there is high interest in disrupting HIF pathway, either proximally (by direct HIF inhibitors) or distally (by inhibiting effector pathways). The goal of proximal inhibition is to shut down all HIFα activity (e.g., by abolishing DNA-binding activity). However, given the many essential roles of HIFs, this strategy may lead to intolerable side effects. In addition, there are three known HIFα/β complexes (i.e., HIF-1α/β, HIF-2α/β, and HIF-3α/β), each with partially redundant functions, and it is technically difficult to abolish the activity of each of these transcriptional factors by small-molecule inhibitors. Instead, an interesting concept has emerged to decrease the translation of HIFα (see below). Ideal distal inhibition involves identifying and blocking key HIF effectors, including those involved in cell proliferation (TGF-β, VEGF-A, and PDGFβR) and metabolism (Glut1) by a combination of agents (e.g., by blocking receptor-ligand interaction). This strategy would seemingly involve treatment with multiple small-molecule inhibitors with potentially additive toxicity. Recently, there has been increasing interest in multitargeted tyrosine kinase inhibitors, which have shown impressive clinical efficacy with tolerable side effects. Because it is difficult (if not impossible) to independently achieve either complete proximal or complete distal inhibition of the HIF pathway, it is likely that future therapy will involve multiple agents, some that target HIF directly combined with others that block key effector molecules.

Distal inhibitors

Sunitinib. Sunitinib (Sutent, Pfizer, New York, NY) is an orally bioavailable small molecule that inhibits multiple split kinase domain RTKs in tissue culture experiments [RTK, including VEGF receptor (VEGFR) 1 and VEGFR2, PDGF receptor (PDGFR) α and PDGFRβ, KIT receptor, and FLT3 receptor] and mice by competitively binding with ATP at the tyrosine kinase active site (14). Thus, sunitinib potentially inhibits several distal effectors that contribute to the pathogenesis of RCC, including VEGF-A and PDGF. In a phase I trial, sunitinib showed partial response to several tumors including RCC and gastrointestinal stromal tumors (15). A regimen of 50 mg daily for 4 weeks followed by 2 weeks off was evaluated in two separate phase II studies in patients with metastatic RCC and progressive disease while on cytokine-based immunotherapy. In the first trial of 63 patients, 25 (40%) achieved partial responses with sunitinib and an additional 17 (27%) had stable disease lasting 3 months or longer; thus, ~70% of patients benefited. Median time to tumor progression was 8.7 months (95% confidence interval, 5.5-10.7) and median overall survival was 16.4 months (16). A second phase II trial was conducted in clear cell metastatic RCC and confirmed the antitumor activity and safety observed in the first phase II trial. Following a median of 7 months of therapy in 105 evaluable patients, an independent third-party assessment showed 36 patients with partial response (34%; 95% confidence interval, 25-44) and a median progression-free survival of 8.3 months (95% confidence interval, 7.8-14.5 months; ref. 17).

A large (750 patients) randomized, multicenter phase III trial comparing sunitinib to IFNα in first-line treatment of clear cell metastatic RCC is ongoing. Recent preliminary analysis showed unprecedented activity by sunitinib as a monotherapy for metastatic RCC. Following randomization of 375 patients to sunitinib and 375 patients to IFNα, preliminary analysis (by third-party independent review) showed median progression-free survival of 47.3 weeks for sunitinib versus 22.0 weeks for IFN-α (hazard ratio, 0.415; \( P < 0.0001 \)) and a partial response rate of 31% for sunitinib versus 6% for IFN-α (\( P < 0.0001 \)). The partial response rate by investigator assessment was 37% for sunitinib versus 9% for IFN-α (\( P < 0.000001 \)). These results showed significant improvement in progression-free survival and an objective response rate for sunitinib over IFN-α in first-line treatment of metastatic RCC. The toxicity profile of sunitinib relative to IFN-α was comparable. Thus, sunitinib is now regarded as the standard for first-line treatment of metastatic RCC.

Sorafenib. Sorafenib (Nexavar; Bayer Corp., West Haven, CT and Onyx Pharmaceuticals, Emeryville, CA) is an orally bioavailable small molecule in the class of bis-aryl ureas that was initially found to potentally inhibit the serine/threonine Raf-1 kinase (which phosphorlates proteins b-raf and c-raf), in tissue culture and mice experiments. It has also subsequently been found to inhibit several RTKs, including VEGF and PDGFRs (18). Sorafenib was studied in a large phase II randomized discontinuation trial involving >500 patients with various solid tumors, including 202 with metastatic RCC. Following 12 weeks of therapy, 73 metastatic RCC patients had tumor shrinkage >25%, and 65 patients had stable disease (<25% tumor shrinkage and <25% tumor growth). These 65 patients were randomized at week 12 to therapy with either placebo (\( n = 33 \)) or continuation of sorafenib (\( n = 32 \)). The median progression-free survival from randomization was significantly longer with sorafenib (24 weeks) compared with placebo (6 weeks; ref. 19). A large, randomized phase III trial comparing sorafenib with placebo was initiated involving 900 patients with treatment-refractory metastatic clear cell RCC (\( n = 451 \) sorafenib arm; \( n = 452 \) placebo arm; ref. 20). Interim analysis showed the median duration of progression-free survival to be 24 weeks in sorafenib patients compared with 12 weeks in the placebo group (hazard ratio, 0.44; \( P < 0.000001 \)). Interim analysis (by independent review) showed 80% overall response in the sorafenib arm (2% with partial response and 78% with stable disease) compared with 55% in the placebo arm (0% partial response and 55% stable disease). As of November 2005, the median overall survival was 19.3 months with sorafenib and 15.9 months with placebo, although this did not attain a level of significance at this interim analysis (21).

Bevacizumab (Avastin) is a humanized recombinant monoclonal antibody that binds VEGF-A, thus blocking its interaction with the VEGFR1 and VEGFR2. A phase II trial comparing placebo (\( n = 40 \)), low-dose bevacizumab (4.5 mg/kg loading dose, 3 mg/kg on day 7 and every 2 weeks thereafter; \( n = 37 \)), and high-dose bevacizumab (15 mg/kg loading dose, 10 mg/kg on day 7 and every 2 weeks thereafter; \( n = 39 \)) showed modest partial response (10%, four patients)
with the high-dose regimen. Median time to progression was 2.5 months with placebo, 3.0 months with low-dose bevacizumab, and 4.8 months with high-dose bevacizumab (22). Thus, anti-VEGF-A therapy results in a less pronounced effect relative to the multitargeted approach. Because of this modest partial response, bevacizumab monotherapy has a limited role; current combination trials are under way with sunitinib (phase I/II), as well as with IFN, and results are pending.

Proximal inhibitors

**Temsirolimus.** Temsirolimus (CCI779, Wyeth Pharmaceuticals, Cambridge, MA) targets mammalian target of rapamycin (mTOR), a serine/threonine kinase involved in the phosphatidylinositol 3-kinase/AKT pathways. On activation of this pathway, mTOR phosphorylates a component of the mRNA translation initiation complex, resulting in increased translation of proteins involved in cell cycle progression, in addition to increased translation of HIFα. Temsirolimus forms a complex with FK-506-binding protein-12, and this complex inhibits mTOR kinase activity (23) and decreases HIFα level (24).

In a randomized phase II trial, 111 patients with advanced, refractory RCC were treated with three different dose levels of temsirolimus (25.0, 75.0, and 250 mg) given i.v. (25). Seven percent of patients achieved a partial or complete response; no significant differences in outcome were noted between dose levels. The median time to progression was 5.8 months, with median survival for the entire population was 15.0 months. A recent phase III randomized trial compared single-agent temsirolimus (25 mg) versus temsirolimus (15 mg) plus IFNα versus single-agent IFNα as first-line treatment in 626 patients with poor-risk features (as defined by modified Memorial Sloan-Kettering Cancer Center RCC risk criteria). The median survival was significantly improved for temsirolimus (10.9 months) versus IFN (7.3 months; \( P = 0.0069; \) hazard ratio, 0.73, favoring temsirolimus); median survival of temsirolimus plus IFN was 8.4 months (26). This early success of temsirolimus suggests that proximal inhibition of the HIFα pathway is a viable strategy.

**Everolimus.** Everolimus (RAD001, Novartis Pharma AG, East Hanover, NJ) is a serine/threonine kinase inhibitor of mTOR, with an identical mechanism to temsirolimus. A potential advantage of RAD001 is that it can be administered orally. A phase II trial in patients with metastatic RCC is ongoing, with a target accrual of 40 patients. An analysis of the first 25 patients (most of whom were treated previously with and found refractory to interleukin-2 or IFN therapy) administered RAD001 (10 mg) daily showed that 36% \( (n = 9) \) achieved a partial response, 44% had stable disease, and 20% \( (n = 5) \) had progression of disease (27). The clinical role of RAD001 will be further addressed in a randomized trial of patients who develop progressive disease following tyrosine kinase inhibitor therapy.

Some Remaining Questions

Characterization of the VHL and HIF pathways has led to a greater understanding of RCC biology, with subsequent development of effective therapy. In addition, given the up-regulation of HIF pathway in numerous cancers, these agents are currently being tested in many other malignancies. Major mechanistic questions about RCC therapy remain. It is unclear why specific individuals (~80% in phase II and III trials) respond to sunitinib or sorafenib (either stable disease or partial response), whereas others are refractory (~20%). Specifically, it is not known if the response to sunitinib is related to the level of activated VEGF R2 and/or PDGFR. Interestingly, 80% of RCC patients are thought to have VHL/HIF-VEGF/PDGF axis activation; do these patients predictably have the best response to sunitinib? A preliminary analysis of patients treated with either sunitinib, a related compound (AG013736), or bevacizumab plus IFN suggested that patients with VHL loss-of-function exhibited longer duration of response versus those with normal VHL (time to progression, 13.3 versus 7.4 months; \( P = 0.06; \) ref. 28). A disadvantage of this study is that it included diverse agents with different mechanisms. Future pharmacodynamic correlating studies, preferable with single-agent therapy, are required to identify pathway(s) responsible for drug response. These studies will allow for identification of a patient subpopulation that would benefit from therapy optimization strategies.

It is unclear whether the major effect of these targeted agents is inhibition of tumor cells or the surrounding stromal/endothelial cells. For example, sunitinib can potentially block both autocrine VEGF/PDGF stimulation, causing direct cytotoxicity, and inhibit paracrine stimulation, leading to arrested stromal/endothelial development. Similarly, temsorilimus can potentially suppress HIFα within RCC cells, as well as potentially block VEGF/PDGFr-stimulated HIFα production within cancer stroma. Sunitinib is directly tumoricidal in gastrointestinal stromal tumor, leading to necrosis within several weeks of treatment. We have observed similarly that patients responding to sunitinib generally exhibit evidence of tumor necrosis on computed tomography scans within the first few treatment cycles. We continue efforts to dissect the contributions of tumoricidal versus antistromal effects of sunitinib.

Lastly, the clinical response to these agents is not permanent. Rather, the time to progression following VHL/HIF/VEGF axis targeted therapy is, on average, approximately 6 to 12 months. Tumors adapt; the genes and mutations responsible for this adaptation/resistance are unknown. One thought is that, because resistance occurs, these agents may not solely act on normal endothelial and stromal cells to block angiogenesis. Rather, these agents may act directly on the evolving cancer cells (e.g., to block proliferation signals), and over time, the tumors mutate and evolve. Consistent with this hypothesis, resistance to other RTK inhibitors in diverse tumors (e.g., imatinib in chronic myelogenous leukemia and erlotinib in non–small cell lung cancer) can occur via active site RTK mutations within tumor cells, such that the binding affinity of the inhibitor is diminished. Alternatively, genetic instability of cancer cells may lead to a higher level of multiple angiogenic factors, and assuming that the major action of the drugs is to block endothelial/stromal development, cancer cells may adapt by simply releasing higher concentration of growth factors. Comprehensive studies to
address mechanisms of resistance are required to reconcile the competing theories.

In summary, elucidation and characterization of the VHL-HIF-VEGF axis has translated into development of therapies with improved clinical response. In the near future, we will conduct preclinical experiments and formal clinical trials on combinations of proximal and distal HIF inhibitors. In addition, we will strive to identify characteristics (e.g., presence of activated VEGFR2) within patient subpopulation that most benefit from therapy. We hope this will further optimize of multianti for RCC patients. Ultimately, prolonged substantial improvement in disease-free survival will likely require identification of other pathways that contribute to RCC pathogenesis. For example, it was found recently that VHL binds and stabilizes p53, thus preventing Mdm2-mediated ubiquitination (29). This leads to a further question: does lack of p53 function in loss-of-function VHL-associated RCC contribute to the chemotherapy-resistant trait of this disease?

Evolution of RCC likely results from multiple mutations, and HIF/VHL–independent pathways that are dysregulated need to be characterized. In the inherited VHL syndrome, mutated VHL results in multiple bilateral renal cysts; however, progression to RCC may depend on additional DNA changes. Several lines of investigations show that additional pathways may be important during clear cell RCC evolution. Comparative genome hybridization analysis of RCC samples showed that, on average, there are 15 genomic gains/losses, in addition to frequent chromosome 3p loss (30). Our preliminary analysis on matched-paired primary and metastatic lesions by array comparative genome hybridization analysis showed that additional genetic changes occur during metastasis. Lastly, analysis of RCC occurring as a secondary malignancy (following chemotherapy) showed characteristic translocations (t(X;17)(p11;q25) and t(X;1)(p11;q21)) resulting in gene fusion products that express a MiTF/TFE family of transcription factors (31). These and other lines of investigations will most likely identify additional pathways amenable to therapeutic intervention.

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