

Renal cell carcinoma

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Purpose of review

This review focuses on recent developments in the biology and clinical therapeutics of renal cell carcinoma. Given historically limited advances in this disease, a more thorough understanding and testing of rationally targeted agents is needed.

Recent findings

Von Hippel-Lindau gene inactivation is observed in most clear cell renal carcinoma, driving the malignant phenotype. The resulting vascular endothelial growth factor overexpression has been targeted through various approaches, with a clear signal of anti-tumor activity. In addition, immunotherapy remains a therapeutic standard in renal cell carcinoma and an area of ongoing investigation. Observation of small renal masses may represent a viable clinical option.

Summary

Renal cell carcinoma has become a model disease for rationally targeted therapeutics based on significant understanding of the underlying biology. Recent advances have increased the potential for meaningful improvements in clinical outcomes for renal cell carcinoma patients.

Keywords

hypoxia inducible factor, immunotherapy, vascular endothelial growth factor, von Hippel-Lindau

Introduction

Accumulating knowledge of the underlying biology of renal cell carcinoma (RCC) has led to a greater understanding of this disease. Importantly, this biology is being translated into treatment strategies with the potential to impact clinical outcome. Recent observational studies of small renal masses have better defined the natural history and potential for expectant management. Immunotherapy remains an active area of investigation based on modest clinical benefit and generation of relevant immune responses in vaccination trials. Further, novel therapeutics directed against vascular endothelial growth factor (VEGF) have demonstrated substantial anti-tumor effects in initial trials. This review will examine recent publications relevant to RCC biology and clinical management.

Molecular biology of renal cell carcinoma

Substantial gains have been made in clarifying the genetics of renal cell carcinoma in recent years, particularly in tumors with clear cell histology. A majority of patients with RCC have an inactivated von Hippel-Lindau (VHL) tumor suppressor gene [1–6]. The effect of this event is deregulated expression of one or both of a family of hypoxia inducible factors (HIF1 α and HIF2 α) [7]. Mutations that disrupt the tuberous sclerosis complex and other evolving pathways associated with familial hamartoma syndromes also result in dysregulation of HIF2 α and VEGF expression that is mediated through unregulated activation of mTOR [8,9,10]. It was recently reported, however, that the HIF factors induce transcription of an overlapping, but not identical set of genes involved in the cellular response to oxygen (O₂) deprivation. Specifically, in an expression array analysis of cells expressing exclusively HIF2 α , as compared with cells expressing both HIF1 α and HIF2 α , it was observed that HIF2 α has no effect on the expression of glycolytic enzymes [11]. This represents a potentially important distinction in the metabolic activity signature of such tumors. Novel targets of HIF activation were also identified in this screen including a protein called ADRP, a lipid transporter normally expressed in adipose tissue. Interestingly, the expanding list of targets now accounts for much of the unique attributes of conventional renal cell carcinoma: ADRP, neutral lipid accumulation and clear cell histology; VEGF and other angiogenic factors, vascularity; erythropoietin, paraneoplastic polycythemia; IL-6, paraneoplastic fever (Fig. 1).

As the distinct profiles of HIF1 α and HIF2 α emerge, the expression patterns of these factors have also been appreciated. Individual tumors may express only HIF2 α or both factors. The absolute role of either factor is not yet clear.

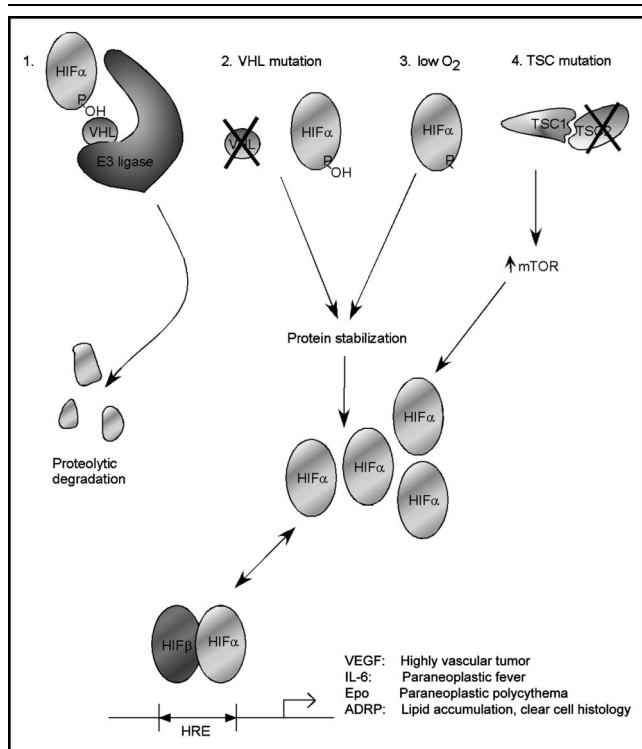
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Figure 1. Mechanism of HIF protein induction and target gene activation

(1) Proteolytic degradation under normal oxygen conditions. (2) HIF accumulation due to VHL loss of function. (3) HIF accumulation due to hypoxic stress. (4) HIF accumulation due to disruption of the TSC complex in tuberous sclerosis.

It has been recently demonstrated that suppression of HIF2 α in a renal carcinoma cell line with RNAi was sufficient to inhibit tumor formation in a xenograft assay [12,13] thus establishing HIF2 α as a factor with oncogenic potential. Although most of the early attention in this pathway was focused on the first of these family members to be identified, the implications of HIF1 α — versus HIF2 α -dominant expression in a given tumor remains uncertain.

As our understanding of the genetics behind RCC expands, so does our ability to use molecular markers as indicators of disease prognosis. Much attention has focused on a subset of genes induced in response to HIF activation. One of these genes is carbonic anhydrase IX (CA IX) [14]. This gene may play a role in regulating the intracellular pH during periods of hypoxic stress. Tissue microarray profiling has implicated this target of HIF expression as an important molecular prognostic factor in conventional renal cell carcinoma [15,16]. Immunostaining for the CAIX (also called G250) protein may provide a potentially straightforward molecular prognostication nomogram, which could be implemented into current clinical prognostic schemas. In addition, evaluation of

renal cell carcinoma signatures by gene expression and other array-based profiling continues to be a robust method for histologic classification of the tumors. A recent genome microarray analysis distinguished between the three most common subtypes of RCC (clear cell, papillary, and chromophobe) in 99% of cases [17]. Prospective validation of genes identified by such genomic analyses may enhance histopathological classification, build upon existing predictive and prognostic clinical schemas and, perhaps most importantly, identify therapeutic targets.

Management of small renal masses

The incidence of asymptomatic renal masses has increased in recent years, in large part due to the widespread use of abdominal imaging. Accepted management of small renal masses (generally defined as masses <4 cm in diameter) has been radical or partial nephrectomy with diagnostic and therapeutic intent. Up to 30% of such masses, however, may not represent malignancy upon pathologic examination [18]. Further, patients with significant medical comorbidities may not be surgical candidates. Thus, complementary strategies for management of small renal masses may permit application of nephrectomy to a more appropriately enriched population. Such a strategy may limit surgery for both non-cancerous renal masses and small RCC tumors unlikely to affect patient survival and also for the medically unfit. Prior small, retrospective series have described the limited growth of small renal masses and the low risk of metastatic spread [19,20]. Three recent series have reported the natural history of small renal masses (Table 1) [21,22,23]. In general, masses were incidental, asymptomatic renal lesions that were not initially surgically addressed because of advanced patient age/comorbidities or patient preference. Variable follow-up imaging was performed and tumor growth rate calculated by tumor diameter and/or volume. Surgery was ultimately performed on some patients, either because of tumor growth or patient request. The growth rate was variable with many patients demonstrating no growth or even tumor regression. Limited patient numbers preclude making a definitive correlation between growth rate and tumor grade. No patient in any series developed metastases or died of RCC. Although additional prospective data on larger number of patients is needed, these results support expectant management as an acceptable initial strategy in selected patients with small renal masses including those unfit or unwilling to undergo surgery. Additional tumor characteristics such as gene or protein expression and radiographic appearance should be investigated to provide complementary data to growth rate in the management strategy of these masses.

Pending further investigation of expectant management as well as the emerging technologies of cryotherapy and radiofrequency ablation, surgical removal of small renal masses remains the standard of care. Non-surgical treatment

Table 1. Recent series describing natural history of small renal masses

Series	Initial mass characteristics	Study features	Median growth rate	Follow-up
Kassouf [22] (n = 24)	Median 2.6 cm in diameter (9.9 cc volume) 22 solid masses 2 Bosniak IV cysts	Retrospective Median follow-up 24 mos. (range, 8–86)	0.49 cm (7.3 cc)/year*	Four pts underwent surgery revealing 4 RCC tumors
Kato [23] (n = 18)	Median 2.0 cm in diameter All solid masses	Retrospective Median follow-up 22.5 mos (range, 12–63)	0.42 cm/year	All 18 pts underwent surgery revealing 18 RCC tumors
Volpe [21*] (n = 29)	Median 2.4 cm in diameter (7.0 cm ³ volume) 22 solid masses 3 Bosniak III cysts 4 Bosniak IV cysts	Prospective Median follow-up 27.9 mos (range, 5–143)	0.1 cm ³ /year	Eight pts underwent surgery revealing 8 RCC tumors, 1 oncocytoma Growth rate not associated with tumor size or appearance (solid vs. cystic) 8 tumors (25%) had significant slope of growth over time

*Calculated based only on 5 tumors that increased in size during observation period.

of such patients should be reserved for patients on a clinical trial until more definitive data are available.

Cytokine immunotherapy

Immunotherapy has been the mainstay of treatment for renal cell carcinoma for more than 20 years and continues to evolve. The use of the cytokine agents interferon alpha and interleukin-2 (IL-2), in addition to traditional use as monotherapy for renal cell carcinoma, continue to play a role in investigational combination regimens. Thalidomide has been of particular interest for some time due to potential angiogenic effects. In a recent first-line phase II trial of thalidomide and interferon-alpha an overall response rate of 7% was reported, with an additional one third of patients requiring discontinuation of thalidomide owing to excessive toxicity [24]. Additionally, interim analysis of a randomized phase III study of interferon-alpha plus thalidomide versus interferon-alpha alone presented at ASCO 2004 demonstrated similarly discouraging results, with the combination therapy arm failing to demonstrate an advantage over interferon-alpha monotherapy in response rate, quality of life, or overall survival [25*]. Cytokines, however, will continue to provide the foundation for investigational combinations, especially with novel molecularly targeted therapies, as discussed elsewhere in this review. Phase I evaluation of interferon-alpha in combination with IL-12, a cytokine gaining interest in this disease, was found to be tolerable, with primary dose limiting toxicity limited to grades 3 and 4 hepatotoxicity and neutropenia/leukopenia [26]. Unique methods of delivering cytokine therapy are also undergoing investigation, in particular, studies of percutaneous CT-guided intratumoral immunotherapy show this mechanism to be technically feasible with relative safety, but the efficacy of this approach remains unknown. Advances in molecular biology

described above may also aid in the optimal delivery of cytokines. Expression of carbonic anhydrase IX (G250), a VHL-mediated protein, was recently demonstrated to predict response to high-dose IL-2 [15,27*]. Further prospective validation of this observation may allow enrichment of the RCC population for IL-2 response. Investigation of this or other predictive markers with low-dose cytokine therapy or other targeted therapy is of interest.

Cellular immunotherapy

Non-cytokine based immunotherapy continues to be an area of active investigation. Nonmyeloablative transplantation is one mechanism of inducing an immune response in the form of graft versus host disease. A recent report by Igarashi *et al.* [28] identified *in vitro* cytotoxic activity of allogeneic NK cells in renal carcinoma cells as a potential mechanism of anti-tumor activity in this modality. A comprehensive review of the pilot trials of allogeneic transplantation demonstrates an important proof of principle that an anti-tumor immune response can be demonstrated in the donor compartment [29]. Many questions regarding this therapy remain, including the optimal protocol of chemotherapy conditioning, the utility of post-transplant donor lymphocyte infusion, and the choice of patients for transplant. Foremost, the absolute benefit of this modality of therapy remains uncertain in the population of patients with advanced renal cell carcinoma, and therefore remains limited to clinical investigations. An additional modality of immunologic treatment undergoing investigation is the expanding strategies of dendritic cell tumor vaccination in all stages of disease. Currently, these strategies are highly specialized and vary tremendously based on the site preparing the vaccine, but have largely coalesced to methods using dendritic cell (DC) carriers of a varied host of tumor antigens. DCs are potent antigen presenting

cells and can be incorporated into vaccines by introducing peptides, proteins, RNA, or genes as a source of specific antigens. In addition, whole tumor cells can provide the antigen from tumor cell lysates or the tumor cells can be used to form cellular fusions with the DCs for broad spectrum anti-tumor immune response [30]. A European phase III randomized trial of adjuvant vaccination with an autologous renal tumor cell vaccine in patients with resected T2-3b pN0-3 M0 tumors showed a benefit in 5-year progression-free survival (77.4% in the treated group and 67.8% in the control group) [31]. No overall survival advantage has yet been demonstrated. The vaccine was well tolerated with minimal adverse events. Additional validation of this approach is needed, however, before adopting this modality as standard adjuvant treatment. In the metastatic setting, one vaccine in development incorporated an allogeneic tumor lysate-pulsed DC product [32]. This investigation also showed no significant toxicity to the vaccine or the adjuvant drug, hemoecyanin, used in this investigation. Additionally, an immune response was identified by ELISPOT analysis of IFN γ expression and expression of T helper type 1 cytokines. In another vaccine strategy in the metastatic setting, irradiated whole tumor was admixed with GM-CSF as the vaccine platform. This pilot study demonstrated the expected safety immune profile as well as increases in T cell pools consistent with the expected immune response [33]. Vaccination in the setting of renal cell carcinoma demonstrates the generation of potentially relevant immune responses, and remains an active area of study with many remaining issues including the feasibility of centralizing vaccine production and the optimal clinical setting for this treatment modality.

Novel therapeutics

Novel, targeted therapy in metastatic RCC has witnessed an explosion of therapeutic approaches in the past year. Most of these are directed against VEGF based on the inherent VHL gene inactivation underlying RCC as described above. The resulting VHL gene silencing leads to induction of hypoxia-regulated genes including VEGF, a potent pro-angiogenic protein, and platelet-derived growth factor (PDGF), expressed on pericytes, which provide structural support to endothelial cells [34–41]. Approaches to neutralize the effects of VEGF and PDGF in RCC have thus been explored.

Anti-VEGF antibody

The initial trial of anti-VEGF therapy in metastatic RCC employed bevacizumab, a neutralizing anti-VEGF antibody, in treatment-refractory RCC [42]. One hundred sixteen patients with treatment-refractory, metastatic clear cell RCC were randomized to receive placebo, low-dose (3 mg/kg) bevacizumab or high-dose (10 mg/kg) bevacizumab given intravenously every 2 weeks. Despite a low (10%) objective response rate, a significant prolongation of time to disease progression (TTP) was observed in

the high-dose bevacizumab arm. Further, due to stringent progression criteria employed, several patients came off study for disease progression, but with a lower total tumor burden than baseline. This fact may have allowed underestimation of the ultimate treatment effect of this approach. This trial first identified the anti-tumor potential of anti-VEGF approaches in RCC. Patients with disease progression on placebo in the above study were eligible to enter a separate study and receive low-dose bevacizumab alone or low-dose bevacizumab plus thalidomide escalated intra-patient from 200 mg to 800 mg daily [43]. Twenty-two of the 40 placebo arm patients entered this study; an initial 10 treated with low-dose bevacizumab alone and a subsequent 12 treated with low-dose bevacizumab plus thalidomide. There were no objective responses in either group and no significant difference in progression-free survival between the groups (2.4 months for bevacizumab alone versus 3.0 months for bevacizumab plus thalidomide; $P = 0.63$). Therapy in both arms was generally well tolerated with toxicity as expected for each agent. This trial failed to demonstrate additive or synergistic anti-tumor effects of these agents, but is noteworthy in the design that allowed relatively rapid evaluation of two agents in this setting.

Follow-up data has also been recently reported on patients in the original study [44], providing insight into the feasibility of long-term dosing of bevacizumab in metastatic RCC. Four patients have been undergoing bevacizumab therapy without tumor progression for 3 to 5 years. Two patients completed a protocol-defined 2 years of therapy with tumor shrinkage, demonstrated tumor progression off therapy, and re-attained tumor regression with reinstitution of bevacizumab for an additional 3 to 3.5 years. Two additional patients achieved stable disease with bevacizumab dosing for 4+ years. Long-term toxicity has included primarily nephrotic range proteinuria with normal renal function in three of four patients.

Bevacizumab has been further investigated in combination with an anti-epidermal growth factor receptor (EGFR) strategy. TGF- α is HIF-regulated growth factor for RCC, with biologic effect through interaction with the EGFR [45–47]. Single agent studies, however, with agents directed against the EGFR receptor have demonstrated limited anti-tumor effect [48]. Nonetheless, pre-clinical investigation in human RCC xenograft models of bevacizumab and erlotinib, a small molecule EGFR inhibitor, have demonstrated potential benefit of combination therapy on tumor growth inhibition [49], perhaps because EGFR resistance is mediated through VEGF [50]. A clinical trial in metastatic RCC with bevacizumab 10 mg/kg IV q 2 weeks in combination with erlotinib 150 mg PO QD reported a 25% partial response rate and a 66% stable disease rate [51 \bullet]. A recently completed randomized phase II trial of bevacizumab with or without erlotinib in untreated,

metastatic RCC may provide further insight into potential additive synergistic clinical effect of this combination therapy.

Small molecule vascular endothelial growth factor receptor inhibitors

An alternative approach to VEGF inhibition involves small molecule tyrosine kinase inhibitors. These multi-targeted agents inhibit not only VEGFR, but also the PDGF receptor (PDGFR). Table 2 summarizes the clinical data on anti-VEGF agents in RCC. Comparison of anti-VEGF agents is not currently possible due to the individual studies employing different patient selection, methodology, and response criteria. Nonetheless, clear signs of anti-tumor activity have been observed (both objective responses and tumor regression not meeting criteria for response were recorded as stable disease), placing VEGF blockade strategies at the forefront of RCC clinical investigation.

SU11248

SU11248 (Pfizer, Inc. La Jolla, CA) is an orally bioavailable oxindol small molecule tyrosine kinase inhibitor of VEGFR-2 and PDGFR-B. *In vitro* assays have demonstrated inhibition VEGF-induced proliferation of endothelial cells and PDGF-induced proliferation of mouse fibroblast cells [52]. Investigation in mouse xenograft models demonstrated growth inhibition of various implanted solid tumors and eradication of larger, established tumors [52].

SU11248 has been investigated in a single-arm, multi-institutional phase II of SU11248 in advanced RCC patients failing initial cytokine treatment (n = 63) [53*].

Patients were treated with 50 mg PO daily of SU11248 on a 4 weeks on, 2 weeks off cycle. Fifteen patients (33%) obtained a partial response per RECIST criteria. Of the 15 patients who achieved a partial response, 1 patient has progressed at 5 months and 14 remain progression-free with median duration of response of 6+ months. Therapy was well tolerated in general with grade 1 or 2 fatigue/asthenia (78%), nausea (56%), diarrhea (51%), and stomatitis (44%). Two patients were taken off the study for asymptomatic decreases in left ventricular ejection fraction of >20% compared with baseline. A confirmatory single-arm phase II trial in 100 cytokine-refractory, metastatic RCC patients has recently completed accrual and a randomized phase III trial versus IFN α monotherapy in untreated metastatic RCC patients is ongoing.

BAY 43-9006

BAY 43-9006 (Bayer Pharmaceuticals, West Haven, CT and Onyx Pharmaceuticals, Richmond, CA) is an orally bioavailable bi-aryl urea Raf kinase inhibitor, with demonstrated inhibition of Ras-dependent human tumor xenograft models [54]. Activated Ras promotes cell proliferation through the Raf/MEK/ERK pathway by binding to and activating Raf kinase. BAY 43-9006 has also demonstrated direct inhibition of VEGFR-2, VEGFR-3, and PDGFR-B [55]. Xenograft models treated with daily BAY 43-9006 demonstrated significant inhibition of tumor angiogenesis, as measured by anti-CD31 immunostaining.

A phase II randomized discontinuation study with BAY 43-9006 has been reported in refractory solid tumors, including 112 patients with metastatic RCC [56*]. All patients

Table 2. Summary of clinical results with VEGF targeting agents in metastatic RCC

Agent	Trial design	Clinical activity	Common toxicity
VEGF ^a binding antibody Bevacizumab (Avastin®) [40]	Randomized, placebo-controlled trial 100% pre-treated pts	10% response rate (WHO ^b criteria) Delay of TTP ^c vs. placebo (2.5 vs. 4.9 months)	Hypertension, proteinuria
Bevacizumab + erlotinib [51*]	Single-arm phase II 32% pre-treated	21% response rate (RECIST ^d criteria)	Not reported
VEGF receptor inhibitors SU011248 [53*]	Single-arm phase II 100% pre-treated	33% response rate (RECIST criteria)	Fatigue/asthenia, nausea, diarrhea, stomatitis
PTK787/ZK [57]	Phase I/II 53% pre-treated	5% response rate (WHO criteria)	Nausea, fatigue, vomiting
BAY 43-9006 [56*]	Randomized discontinuation design 86% pre-treated	15% response rate (WHO criteria)	Hand-foot syndrome rash, fatigue, diarrhea, hypertension

^aVascular endothelial growth factor; ^bWorld Health Organization; ^ctime to progression; ^dresponse evaluation criteria in solid tumors.

received oral BAY 43-9006 400 mg BID, and patients with stable disease (defined as tumor burden within 25% of baseline by the sum of the bidimensional measurement of tumors) after 12 weeks of treatment were randomized to continue drug or receive placebo. Patients with $\geq 25\%$ tumor shrinkage at 12 weeks continued open label BAY 43-9006. Of the 89 evaluable RCC patients who had reached the initial 12-week assessment, 13 patients (15%) achieved a partial response and 77% achieved stable disease per WHO criteria. A randomized, placebo-controlled phase III trial in cytokine-refractory RCC patients is ongoing and a similar trial in the adjuvant setting after nephrectomy is planned.

Conclusion

Clinical observers have long noted the variable natural history of renal cell carcinoma, from indolent stability to rapid growth and death from metastases. A growing understanding of the molecular biology has begun to further characterize this variable natural history. From the potential indolence of small renal masses to the rationally targeted therapeutic recently demonstrated to have a significant anti-tumor effect in RCC, it is clear that a more thorough understanding of the molecular basis of this cancer has led, and will continue to lead, to advances in clinical management.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

- 1 Gnarr JR, Tory K, Weng Y, *et al.* Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat Genet* 1994; 7:85–90.
- 2 Shuin T, Kondo K, Torigoe S, *et al.* Frequent somatic mutations and loss of heterozygosity of the von Hippel-Lindau tumor suppressor gene in primary human renal cell carcinomas. *Cancer Res* 1994; 54:2852–2855.
- 3 Wiesener MS, Munchenhagen PM, Berger I, *et al.* Constitutive activation of hypoxia-inducible genes related to overexpression of hypoxia-inducible factor-1alpha in clear cell renal carcinomas. *Cancer Res* 2001; 61:5215–5222.
- 4 Turner KJ, Moore JW, Jones A, *et al.* Expression of hypoxia-inducible factors in human renal cancer: relationship to angiogenesis and to the von Hippel-Lindau gene mutation. *Cancer Res* 2002; 62:2957–2961.
- 5 Herman JG, Latif F, Weng Y, *et al.* Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc Natl Acad Sci USA* 1994; 91:9700–9704.
- 6 Clifford SC, Prowse AH, Affara NA, *et al.* Inactivation of the von Hippel-Lindau (VHL) tumour suppressor gene and allelic losses at chromosome arm 3p in primary renal cell carcinoma: evidence for a VHL-independent pathway in clear cell renal tumorigenesis. *Genes Chromosomes Cancer* 1998; 22:200–209.
- 7 Maxwell PH, Wiesener MS, Chang GW, *et al.* The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999; 399:271–275.
- 8 Brugarolas JB, Vazquez F, Reddy A, *et al.* TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell* 2003; 4:147–158.
- 9 Brugarolas J, Kaelin WG Jr. Dysregulation of HIF and VEGF is a unifying feature of the familial hamartoma syndromes. *Cancer Cell* 2004; 6:7–10. This review describes alternative mechanisms to VHL loss that result in HIF dysregulation.
- 10 Liu MY, Poellinger L, Walker CL. Up-regulation of hypoxia-inducible factor 2alpha in renal cell carcinoma associated with loss of Tsc-2 tumor suppressor gene. *Cancer Res* 2003; 63:2675–2680.
- 11 Hu CJ, Wang LY, Chodosh LA, *et al.* Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. *Mol Cell Biol* 2003; 23:9361–9374.
- 12 Kondo K, Kim WY, Lechpammer M, Kaelin WG Jr. Inhibition of HIF2alpha is sufficient to suppress pVHL-defective tumor growth. *PLoS Biol* 2003; 1:E83.
- 13 Zimmer M, Doucette D, Siddiqui N, Iliopoulos O. Inhibition of hypoxia-inducible factor is sufficient for growth suppression of VHL-/- tumors. *Mol Cancer Res* 2004; 2:89–95.
- 14 Grabmaier K, AdW MC, Verhaegh GW, *et al.* Strict regulation of CAIX(G250/MN) by HIF-1alpha in clear cell renal cell carcinoma. *Oncogene* 2004; 23:5624–5631.
- 15 Lam JS, Belldegrun AS, Figlin RA. Tissue array-based predictions of pathology, prognosis, and response to treatment for renal cell carcinoma therapy. *Clin Cancer Res* 2004; 10:6304S–6309S.
- 16 Kim HL, Seligson D, Liu X, *et al.* Using protein expressions to predict survival in clear cell renal carcinoma. *Clin Cancer Res* 2004; 10:5464–5471.
- 17 Furge KA, Lucas KA, Takahashi M, *et al.* Robust classification of renal cell carcinoma based on gene expression data and predicted cytogenetic profiles. *Cancer Res* 2004; 64:4117–4121.
- 18 Marszalek M, Ponzholzer A, Brossner C, *et al.* Elective open nephron-sparing surgery for renal masses: single-center experience with 129 consecutive patients. *Urology* 2004; 64:38–42.
- 19 Oda T, Miyao N, Takahashi A, *et al.* Growth rates of primary and metastatic lesions of renal cell carcinoma. *Int J Urol* 2001; 8:473–477.
- 20 Bosniak MA, Birnbaum BA, Krinsky GA, Waisman J. Small renal parenchymal neoplasms: further observations on growth. *Radiology* 1995; 197:589–597.
- 21 Volpe A, Panzarella T, Rendon RA, *et al.* The natural history of incidentally detected small renal masses. *Cancer* 2004; 100:738–745. This prospective series generates the hypothesis that a subset of small renal masses may be followed expectantly without detriment.
- 22 Kassouf W, Aprikian AG, Laplante M, Tanguay S. Natural history of renal masses followed expectantly. *J Urol* 2004; 171:111–113; discussion 113.
- 23 Kato M, Suzuki T, Suzuki Y, *et al.* Natural history of small renal cell carcinoma: evaluation of growth rate, histological grade, cell proliferation and apoptosis. *J Urol* 2004; 172:863–866.
- 24 Clark PE, Hall MC, Miller A, *et al.* Phase II trial of combination interferon-alpha and thalidomide as first-line therapy in metastatic renal cell carcinoma. *Urology* 2004; 63:1061–1065.
- 25 Gordon MS, Manola J, Fairclough D, *et al.* Low dose interferon- $\alpha 2b$ (IFN) + thalidomide (T) in patients (pts) with previously untreated renal cell cancer (RCC). Improvement in progression-free survival (PFS) but not quality of life (QoL) or overall survival (OS). A phase III study of the Eastern Cooperative Oncology Group (E2898) ASCO Proceedings. *J Clin Oncol* 2004; 23: abstract #4516. This presentation did not show any advantage (response rate, PFS or OS) to the thalidomide arm in the final analysis, and thus thalidomide combination therapy in RCC remains investigational.
- 26 Alatrash G, Hutson TE, Molto L, *et al.* Clinical and immunologic effects of subcutaneously administered interleukin-12 and interferon alfa-2b: phase I trial of patients with metastatic renal cell carcinoma or malignant melanoma. *J Clin Oncol* 2004; 22:2891–2900.
- 27 Atkins M, McDermott D, Regan M, *et al.* Carbonic Anhydrase IX (CAIX) expression predicts for renal cell cancer (RCC) patient response and survival to IL-2 therapy. *J Clin Oncol* 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition) 2004; 22: 4512. This data identifies a potential marker predictive of response to high-dose IL-2 potentially applying this therapy to a population enriched for response.
- 28 Igarashi T, Wynberg J, Srinivasan R, *et al.* Enhanced cytotoxicity of allogeneic NK cells with killer immunoglobulin-like receptor ligand incompatibility against melanoma and renal cell carcinoma cells. *Blood* 2004; 104:170–177.
- 29 Takahashi Y, Childs RW. Nonmyeloablative transplantation: an allogeneic-based immunotherapy for renal cell carcinoma. *Clin Cancer Res* 2004; 10: 6353S–6359S.
- 30 Avigan D. Dendritic cell-tumor fusion vaccines for renal cell carcinoma. *Clin Cancer Res* 2004; 10:6347S–6352S.
- 31 Jocham D, Richter A, Hoffmann L, *et al.* Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: phase III, randomised controlled trial. *Lancet* 2004; 363:594–599.

- 32 Pandha HS, John RJ, Hutchinson J, *et al.* Dendritic cell immunotherapy for urological cancers using cryopreserved allogeneic tumour lysate-pulsed cells: a phase I/II study. *BJU Int* 2004; 94:412–418.
- 33 Schwaab T, Tretter CP, Gibson JJ, *et al.* Immunological effects of granulocyte-macrophage colony-stimulating factor and autologous tumor vaccine in patients with renal cell carcinoma. *J Urol* 2004; 171:1036–1042.
- 34 Lee JS, Kim HS, Jung JJ, *et al.* Expression of vascular endothelial growth factor in renal cell carcinoma and the relation to angiogenesis and p53 protein expression. *J Surg Oncol* 2001; 77:55–60.
- 35 Takahashi A, Sasaki H, Kim SJ, *et al.* Markedly increased amounts of messenger RNAs for vascular endothelial growth factor and placenta growth factor in renal cell carcinoma associated with angiogenesis. *Cancer Res* 1994; 54:4233–4237.
- 36 Hemmerlein B, Kugler A, Ozisik R, *et al.* Vascular endothelial growth factor expression, angiogenesis, and necrosis in renal cell carcinomas. *Virchows Arch* 2001; 439:645–652.
- 37 Tomisawa M, Tokunaga T, Oshika Y, *et al.* Expression pattern of vascular endothelial growth factor isoform is closely correlated with tumour stage and vascularisation in renal cell carcinoma. *Eur J Cancer* 1999; 35:133–137.
- 38 Igarashi H, Esumi M, Ishida H, Okada K. Vascular endothelial growth factor overexpression is correlated with von Hippel-Lindau tumor suppressor gene inactivation in patients with sporadic renal cell carcinoma. *Cancer* 2002; 95:47–53.
- 39 Nicol D, Hii SI, Walsh M, *et al.* Vascular endothelial growth factor expression is increased in renal cell carcinoma. *J Urol* 1997; 157:1482–1486.
- 40 Na X, Wu G, Ryan CK, *et al.* Overproduction of vascular endothelial growth factor related to von Hippel-Lindau tumor suppressor gene mutations and hypoxia-inducible factor-1 alpha expression in renal cell carcinomas. *J Urol* 2003; 170:588–592.
- 41 Kourembanas S, Hannan RL, Faller DV. Oxygen tension regulates the expression of the platelet-derived growth factor-B chain gene in human endothelial cells. *J Clin Invest* 1990; 86:670–674.
- 42 Yang JC, Haworth L, Sherry RM, *et al.* A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003; 349:427–434.
- 43 Elaraj DM, White DE, Steinberg SM, *et al.* A pilot study of antiangiogenic therapy with bevacizumab and thalidomide in patients with metastatic renal cell carcinoma. *J Immunother* 2004; 27:259–264.
- 44 Yang JC. Bevacizumab for patients with metastatic renal cancer: an update. *Clin Cancer Res* 2004; 10:6367s–6370s.
- 45 Knebelmann B, Ananth S, Cohen HT, Sukhatme VP. Transforming growth factor alpha is a target for the von Hippel-Lindau tumor suppressor. *Cancer Res* 1998; 58:226–231.
- 46 Gunaratnam L, Morley M, Franovic A, *et al.* Hypoxia inducible factor activates the transforming growth factor-alpha/epidermal growth factor receptor growth stimulatory pathway in VHL(-/-) renal cell carcinoma cells. *J Biol Chem* 2003; 278:44966–44974.
- 47 de Paulsen N, Brychzy A, Fournier MC, *et al.* Role of transforming growth factor-alpha in von Hippel-Lindau (VHL)(-/-) clear cell renal carcinoma cell proliferation: a possible mechanism coupling VHL tumor suppressor inactivation and tumorigenesis. *Proc Natl Acad Sci USA* 2001; 98:1387–1392.
- 48 Motzer RJ, Amato R, Todd M, *et al.* Phase II trial of antiepidermal growth factor receptor antibody C225 in patients with advanced renal cell carcinoma. *Invest New Drugs* 2003; 21:99–101.
- 49 Shen BQ, Metz T, Fiebig HH, *et al.* Effect of combination therapy targeting both VEGF and EGF receptor signaling pathways in human renal cell carcinoma explant models. *AACR Proceedings* 2004; 45: abstract #3007.
- 50 Vilorio-Petit A, Crombet T, Jothy S, *et al.* Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies in vivo: a role for altered tumor angiogenesis. *Cancer Res* 2001; 61:5090–5101.
- 51 Hainsworth JD, Sosman JA, Spigel DR, *et al.* Phase II trial of bevacizumab and erlotinib in patients with metastatic renal carcinoma (RCC). *ASCO Proceedings*, Abstract #4502. *J Clin Oncol* 2004; 23.
- This data identified the possible additive or synergistic effect of this combination therapy in RCC.
- 52 Mendel DB, Laird AD, Xin X, *et al.* In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 2003; 9:327–337.
- 53 Motzer RJ, Rini BI, Michaelson MD, *et al.* SU011248, a novel tyrosine kinase inhibitor, shows anti-tumor activity in second-line therapy for patients with metastatic renal cell carcinoma: results of a phase II trial. *ASCO Proceedings*, Abstract #4500. *J Clin Oncol* 2004; 23.
- This data established the substantial anti-tumor activity of small molecule anti-VEGF agents in RCC.
- 54 Lyons JF, Wilhelm S, Hibner B, Bollag G. Discovery of a novel Raf kinase inhibitor. *Endocr Relat Cancer* 2001; 8:219–225.
- 55 Wilhelm S, Carter C, Tang, *et al.* BAY 43-9006 exhibits broad spectrum anti-tumor activity and targets raf/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *AACR Proceedings* 2003; abstract #78.
- 56 Ratain MJ, Flaherty KT, Stadler WM, *et al.* Preliminary antitumor activity of BAY 43 43-9006 in metastatic renal cell carcinoma and other advanced refractory solid tumors in a phase II randomized discontinuation trial (RDT). *ASCO Proceedings*, Abstract #4501. *J Clin Oncol* 2004; 23.
- This data further established the substantial anti-tumor activity of small molecule anti-VEGF agents in RCC, noteworthy for the trial design that may demonstrate a significant disease-stabilizing effect of this agent.
- 57 George D, Michaelson MD, Oh WK, *et al.* Phase I study of PTK787/ZK222584 (PTK/ZK) in metastatic renal cell carcinoma. *ASCO Proceedings*, Abstract #1548. *J Clin Oncol* 2003; 22.