Chromosome 3p Loss–Orchestrated VHL, HIF, and Epigenetic Deregulation in Clear Cell Renal Cell Carcinoma

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Published at jco.org on October 29, 2018.

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0732-183X/18/3636w-3533w/\$20.00

ABSTRACT

Clear cell renal cell carcinoma (ccRCC) is the most common renal cell carcinoma subtype, and metastatic ccRCC is associated with 5-year survival rates of 10% to 20%. Genetically, ccRCC originates from sequential losses of multiple tumor suppressor genes. Remarkably, chromosome 3p loss occurs in more than 90% of sporadic ccRCCs. This results in concurrent one-copy loss of four tumor suppressor genes that are also mutated individually at high frequency in ccRCC (ie, *VHL*, 80%; *PBRM1*, 29% to 46%; *BAP1*, 6% to 19%; and *SETD2*, 8% to 30%). Pathogenically, 3p loss probably represents the first genetic event that occurs in sporadic ccRCC and the second genetic event in *VHL*-mutated hereditary ccRCC. VHL constitutes the substrate recognition module of the VCB-Cul2 E3 ligase that degrades HIF1/2 α , whereas PBRM1, BAP1, and SETD2 are epigenetic modulators that regulate gene transcription. Because 3p loss and VHL inactivation are nearly universal truncal events in ccRCC, the resulting HIF1/2 signaling overdrive and accompanied tumor hyper-vascularization probably underlie the therapeutic benefits observed with vascular endothelial growth factor receptor inhibitors, including sorafenib, sunitinib, pazopanib, axitinib, bevacizumab, cabozantinib, and lenvatinib. Furthermore, recent marked advances in ccRCC genomics, transcriptomics, proteomics, metabolomics, molecular mechanisms, mouse models, prognostic and predictive biomarkers, and clinical trials have rendered invaluable translational insights concerning precision kidney cancer therapeutics. With an armamentarium encompassing 13 drugs that exploit seven unique therapeutic mechanisms (ie, cytokines, vascular endothelial growth factor receptor, mTORC1, cMET/AXL, fibroblast growth factor receptor, programmed cell death-1 and programmed death-ligand 1, and cytotoxic T-cell lymphocyte associated-4) to treat metastatic renal cell carcinoma, one of the imminent clinical questions concerning care of patients with metastatic ccRCC is how a personalized treatment strategy, through rationally combining and sequencing different therapeutic modalities, can be formulated to offer the best clinical outcome for individual patients. Here, we attempt to integrate recent discoveries of immediate translational impacts and discuss future translational challenges and opportunities.

J Clin Oncol 36:3533-3539. © 2018 by American Society of Clinical Oncology

INTRODUCTION

Renal cell carcinoma (RCC) encompasses more than 10 different histopathologic subtypes. Clear cell RCC (ccRCC) is most prevalent (approximately 75%) and accounts for approximately 80% of kidney cancer fatalities.¹ Kidney cancer treatment has evolved dramatically over the past decade from two (around 2004) to 13 (around 2018) US Food and Drug Administration–approved drugs, representing seven distinct effective treatment mechanisms encompassing targeted and immunotherapeutic agents.^{1,2} At the same time, basic knowledge concerning ccRCC pathobiology has greatly expanded beyond a single mutation of the von Hippel-Lindau (*VHL*) tumor suppressor gene (TSG).^{1,3}

Inactivation of *VHL* and chromosome 3p loss were the only two known oncogenic driver events in ccRCC before international large-scale cancer genomics sequencing campaigns.⁴ Sequencing of primary ccRCC tumors demonstrated that *VHL* (approximately 80%), *PBRM1/BAF180* (29% to 46%), *SETD2* (8% to 30%), and *BAP1* (6% to 19%) are the four most commonly mutated genes.⁵⁻⁸ Strikingly, all four TSGs locate at the short arm of chromosome 3 (Fig 1), and one-copy loss of 3p occurs in more than 90% of ccRCCs. Furthermore, these three newly identified 3p TSGs encode epigenetic regulators that modulate gene transcription. PBRM1 protein containing PBAF complex functions as a chromatin remodeling enzyme; the SETD2 protein functions as histone H3 lysine K36 trimethylase

(H3K36TM); and the BAP1 protein functions as a ubiquitin C-terminal hydrolase (Fig 1).⁹ Mutations in *PBRM1*, *SETD2*, and *BAP1* were also detected in non-clear cell RCC.^{10,11}

Unlike many cancers that originate from gain-of-function mutations in oncogenes such as *EGFR* and *RAS*, ccRCC manifests with prevalent loss-of-function mutations in TSGs, making the discovery of predictive biomarkers challenging.⁹ Nevertheless, new clinically relevant patient-derived cell line and xenograft models,^{12,13} novel genetically engineered mouse models,¹⁴⁻¹⁶ and informative outcome-based biomarker⁸ studies have begun to shed light on how seemingly distinct research areas are in fact highly interconnected.³ Here, we wish to update key new translational findings in ccRCC and highlight emerging biomarkers and drugs that could affect future kidney cancer care.

THE VHL-HIF-HYPOXIA-METABOLIC INNER CIRCLE

VHL: VCB-Cullin2 E3 Ligase for HIF α Degradation

The identification of mutations at the *VHL* gene located at 3p25 as the principal genetic event in both hereditary and somatic ccRCC cemented the quintessential role of VHL in kidney cancer pathobiology.⁴ VHL is a multipurpose adaptor protein that serves as the substrate recognition module of the VCB (VHL–elongin C–elongin B)–Cul2 E3 ligase that marks hypoxia-inducible factor (HIF)-1 α and HIF-2 α proteins for degradation.¹⁷⁻¹⁹ Of note, a small number of ccRCC tumors that lack *VHL* mutations have mutations in the *TCEB1/ELOC* gene that encodes elongin C.^{6,20}

HIFs: Oxygen Sensing, Metabolic Adaptation, Mitochondria, Vascularization, and Tumorigenesis

The study of oxygen sensing led to the discovery of the HIF transcription factors.¹⁷⁻¹⁹ Under normal oxygen tension, HIF α is prolyl hydroxylated by EGLN, ubiquitinated by VCB-Cul2, and degraded by 26S proteasome.²¹ Under low oxygen tension such as

vessel injury, HIF α is stable and initiates a myriad of hypoxia-specific transcription programs.¹⁸ Key effects include increased glycolysis, decreased mitochondrial function, and induced neo-vascularization (Fig 2).^{17,18,21} The pathologic loss of VHL in ccRCC results in persistently elevated HIFs, which in turn incurs the classic clear cell morphology and the highly vascular nature observed with ccRCC.^{1,22,23} However, the long latency period (more than 30 years) between diagnosis of VHL syndrome and development of ccRCC and the insufficiency of VHL loss alone to induce ccRCC in mice argue for the necessity of cooperative events.²⁴ Of note, the mouse model does not perfectly match the human experience because targeted loss of *Vhl* does not mimic the heterozygous loss of the other 3p genes seen in humans and, even if loss of chromosome 6 in mice that contains VHL was mimicked, the other genes are on different chromosomes, with *Pbrm1* and *Bap1* being on chromosome 14 and *Setd2* on chromosome 9.

HIF-1 Versus HIF-2: Friends or Foes in Tumorigenesis

HIFs are transcription factors consisting of an invariable HIF-1 β and a variable, oxygen-sensing HIF-1 α , HIF-2 α , or HIF-3 α that form heterodimeric complexes. HIF-1 and HIF-2 function as transcription activators, whereas HIF-3 functions as a repressor because of its lack of a transactivation domain. Shared and unique transcriptional targets of HIF-1 and HIF-2 have been demonstrated, explaining their common roles in hypoxia-induced gene expression and their distinct biologic outputs.^{17-19,22} For example, HIF-1 inhibits Myc targets, whereas HIF-2 activates these targets.²⁵ Because cancers commonly contain hypoxic areas, the role of HIF-1 in human cancer progression has been demonstrated in many different cancer types.¹⁸ However, in ccRCC where VHL is near universally inactivated, the roles of HIF-1 and HIF-2 in tumorigenesis become perplexing.^{18,19} In transgenic mouse models, expression of the constitutive active, nondegradable HIF-1 α but not HIF-2 α mutant in the renal proximal tubule driven by the γ -glutamyl transpeptidase promoter resulted in ccRCC,^{26,27} whereas

Fig 1. Chromosome 3p clear cell renal cell carcinoma tumor suppressor genes and their respective protein complexes.

Fig 2. The oncogenes and tumor suppressor genes operate in renal epithelium to control signaling output and prevent tumorigenesis.

in human ccRCC, one-copy loss of the *HIF-1α* gene through the loss of chromosome 14q and the expression of HIF-2 are associated with clinical progression and poor clinical outcome.^{28,29} One probable scenario to reconcile these seemingly contradictory findings is that the initial expression of HIF-1 is required for ccRCC initiation and the subsequent expression of HIF-2 facilitates disease progression.

CHROMOSOME 3P PATHOBIOLOGY IN ccRCC

Chromosome 3p: The Powerful TSG Cluster

Before the cancer genomics sequencing era, it was already recognized that 3p loss is the dominating karyotypic abnormality in ccRCC.³⁰ The biologic significance of this event in ccRCC was subsequently confirmed yet restricted to the identification of the *VHL* gene on 3p25⁴ and subsequently substantiated with the discovery of three additional prevalently mutated genes in ccRCC—*PBRM1* (29% to 46%), *BAP1* (6% to 19%), and *SETD2* (8% to 30%)—all located at 3p21.^{5,6,8} Hence, four TSGs that play critical roles in maintaining kidney tissue homeostasis become haploinsufficient upon one-copy loss of 3p. Interestingly, in patients with VHL syndrome, 3p loss is the second genetic event,³¹ whereas in somatic ccRCC, 3p loss is the first event and VHL inactivation is the second event.³² Unlike VHL, which has been studied for decades, the tumor suppressor function of *PBRM1*, *BAP1*, and *SETD2* in ccRCC was largely unknown. Here, we briefly summarize recent findings and offer a glimpse into how these three epigenetic TSGs might function in suppressing the pathogenesis of ccRCC.

PBRM1

The SWI/SNF complexes are macromolecular protein complexes that use ATP to mobilize nucleosome, modulate chromatin structure, and thereby regulate central cellular, developmental, and oncogenic processes.³³ There are many different complexes as a result of their interchangeable, dynamic compositions in nature.³⁴ Notably, mutations of individual SWI/SNF subunits are detected and exhibit preferential enrichment in approximately 20% of human cancers.³⁵ PBRM1 is the defining component of the PBAF complex and is most commonly mutated in ccRCC.³⁶

Remarkably, the *in vivo* tumor suppressor role of PBRM1 in ccRCC was confirmed and reported in 2017 by three independent laboratories using three different genetically engineered mouse models where combined losses of VHL and PBRM1 led to multifocal ccRCC in mouse kidney, whereas individual losses did not.¹⁴⁻¹⁶

BAP1

BAP1 (BRCA1-associated protein) was first identified based on its interaction with BRCA1^{37,38} and was recently found to be mutated in multiple cancer types such as mesothelioma, uveal melanoma, and kidney cancer.³⁹ BAP1 is a ubiquitin C-terminal hydrolase, forms complexes (Fig 1), and regulates key cellular pathways, including the cell cycle, cellular differentiation, cell death, gluconeogenesis, and DNA damage response.³⁹ The loss of BAP1 defines a molecular subtype of ccRCC,⁴⁰ occurs near mutual exclusively with the loss of PBRM1,⁴⁰ and portends poor clinical outcome.^{8,41,42} Genetically, the loss of BAP1 in mice results in ccRCC with aggressive morphologic features.¹⁵

SETD2

SETD2 is a histone H3 K36 methyltransferase that regulates chromatin biology and thereby modulates gene transcription and DNA repair.^{43,44} Intriguingly, SETD2 was recently demonstrated to methylate tubulin for cytoskeleton remodeling and STAT1 for interferon response.^{45,46} The prevalence of *SETD2* mutations in ccRCC increases as kidney cancer progresses,^{7,8,47} implicating its function in preventing cancer metastasis. Besides ccRCC, *SETD2* mutations were also detected in papillary RCC and unclassified RCC.^{48,49}

KIDNEY CANCER EVOLUTION

By exploiting integrated multiregional and longitudinal approaches, series of genomic studies demonstrated the presence of conspicuous intratumor heterogeneity in ccRCC through branched evolution (Fig 3).^{32,47,50,51} Phylogenetic study of clonal evolution of ccRCC discovered that the nearly universal loss of 3p (approximately 90%) and common gain of 5q (approximately 67%) in ccRCC⁵ mainly result from a single unbalanced translocation incidence involving chromosomes 3 and 5 through t(3;5) chromothripsis events that occurred decades before cancer diagnosis.³² Furthermore, deterministic evolutionary trajectories that influence primary tumor growth were revealed,⁵¹ and the hallmark genomic drivers of ccRCC metastasis are loss of 9p and 14q where *CDKN2A* and *HIF-1α* reside, respectively.⁵⁰

FUTURE DIRECTIONS

Emerging Biomarkers

The singular loss of VHL is insufficient for initiating ccRCC, which is recognized in both mouse and human VHL loss models.⁵² The ensuing complete loss of PBRM1 leads to the increased transcription output of HIF and STAT targets (Fig 2).¹⁴ Consequently, the dysregulated interplay between HIF and STAT upon combined losses of VHL and PBRM1 creates a feed-forward

Fig 3. Recent analysis using multiregional whole-genome and whole-exome sequencing in a cohort of 100 patients with clear cell renal cell carcinoma (ccRCC) has elucidated a model of tumor evolution for ccRCC. The earliest genetic alteration in the tumorigenic evolution of a sporadic ccRCC was shown to be loss of chromosome 3p, with the most frequent mechanism being an unbalanced translocation resulting in simultaneous loss of chromosome 3p and gain of chromosome 5q with chromothripsis at the breakpoint. This early tumor-initiating cell subsequently develops further genetic alterations to become a ccRCC, and two different groups of evolutionary trajectories were identified based on the sequential gain-of-mutation events, the degree of intratumor heterogeneity, and level of genomic instability. More aggressive evolutionary trajectories were associated with more rapid metastasis to multiple distance sites and poorer overall survival.

amplification loop that maximizes downstream gene expression.¹⁴ Because STATs are key transcription factors in cancer inflammation and immunity,⁵³ the activation of the STAT3 pathway as a result of the combined loss of VHL and PBRM1 could render the resulting tumor prone to immune regulation.¹⁴ Recent approval of single-agent nivolumab (anti-programmed cell death-1 [PD-1] antibody) as second-line therapy and of the combination of ipilimumab (anti-cytotoxic T-cell lymphocyte associated-4 antibody) and nivolumab as first-line therapy for ccRCC has dramatically altered the therapeutic landscape of metastatic kidney cancer.^{2,54} Intriguingly, a recent publication identified PBRM1 loss as a potential genomic biomarker for the treatment response to these immune checkpoint inhibitors.⁵⁵ Altogether, these mechanistic, mouse, and human ccRCC studies implicate a working hypothesis in which the disarmed STAT-PD-1 immunosurveillance can be reactivated through biologic means such as anti-PD-1 and anti-programmed death-ligand 1 (PD-L1) antibodies for therapeutic exploitation (Fig 2). Because *PBRM1*-mutant tumors benefited most from vascular endothelial growth factor receptor (VEGFR) inhibitor treatment, as shown in the RECORD-3 trial comparing sunitinib and everolimus,⁸ the lack of association between *PBRM1* mutations and single-agent therapeutic benefit from the anti-PD-L1 agent atezolizumab, as reported in the IMmotion150 trial comparing atezolizumab and sunitinib, is not totally unexpected. These findings further affirm disrupted VHL-HIF biology as the first quintessential event in ccRCC pathobiology and warrant additional confirmation and refinement with large data sets.

Despite active surveillance after nephrectomy, one third of patients with RCC experience disease progression.¹ Although prognostic clinicopathologic models can predict survival of patients after surgery, a subset of patients (10%) characterized as low risk ultimately die of RCC.⁵⁶ Epigenetic subclassifications of ccRCC offer an opportunity to improve upon clinicopathologic models. Immunohistochemistry (IHC) loss of intranuclear H3K36me3, a surrogate of *SETD2* loss-of-function mutations, is associated with a two-fold increased risk of RCC-specific death in low-risk tumors.⁵⁷ Similarly, loss of BAP1 expression by IHC is associated with a three-fold increased risk of RCC-specific death in low-risk tumors.⁵⁸ Consistent with preclinical models showing increased enhancer of zeste homolog 2 (EZH2) activity during ccRCC invasion and metastases, increased expression of EZH2 by IHC is associated with a six-fold increased risk of RCC-specific death in low-risk tumors.⁵⁹ Given the increasing incidence of small tumors (4 cm or less) as a result, in part, of the increased use of abdominal imaging, these small tumors, which are currently excluded from adjuvant trials, underscore a need to better identify patients with a low-risk prognostic score who have a higher risk of recurrence.

Emerging Therapeutics

With 13 approved drugs exploiting seven effective mechanisms in treating metastatic RCC, the field is quickly moving toward combination trials consisting of one VEGFR inhibitor and one PD-1 or PD-L1 inhibitor. The first success of such a strategy

was reported in the large randomized phase II IMmotion150 trial, which revolutionized treatment of metastatic ccRCC paradigm from single-agent therapy with the VEGFR inhibitor bevacizumab or the anti-PD-1 and anti-PD-L1 agent atezolizumab to a combination of two distinct effective mechanisms.⁶⁰ Because all single-agent VEGFR blockade strategies inevitably encounter resistance and the role of HIF-2 activity in ccRCC aggressiveness has been demonstrated in preclinical and clinical studies, the development of small-molecule inhibitors specifically targeting HIF-2 α and HIF- β , including PT2399 and PT2385, is of clinical significance. In preclinical ccRCC studies, PT2399 exhibited on-target effects in cell-based and xenograft assays,^{61,62} and in a phase I clinical trial, PT2385 exhibited a favorable safety profile and activity in patients with heavily pretreated ccRCC.⁶³ Hence, agents exploiting additional mechanisms of action such as HIF-2 could offer novel treatment modalities for effective combination therapeutic strategies in the future.

APPLIED PATHOLOGY OF CCRCC

Molecular Pathology to Incorporate Multiomics With Histopathology

Recent omic studies have presented an unprecedented, comprehensive molecular understanding of ccRCC pathobiology, which includes genomics, epigenomics, transcriptomics, proteomics, immunogenomics, and metabolomics.^{5,6,23,40,64} Thus far, molecular subtypes based on genomics and transcriptomics are the best studied and most applicable.^{3,65,66} At the chromosomal arm aberration level, commonly detected 5q gain, 9p loss, and 14q loss carry clinical significance.^{28,50} At the somatic mutation level, 18 genes were mutated in more than 5% of patients with in metastatic ccRCC.⁸

Functional Pathology to Link Molecular Pathology With Clinical Outcome

Successful interconnections between molecular pathologies and clinical outcomes are of paramount significance in translation oncology. Notably, the clinical value of individual biomarkers can be context dependent; for example, *PBRM1* mutations detected in localized small ccRCC tumors could be an early indicator for disease progression whereas in advanced ccRCC tumors could be a good prognostic and predictive biomarker.^{7,41,42} In the targeted therapy era, the most important molecular distinction among

metastatic ccRCC is probably mutation status of *PBRM1* and *BAP1* and chromosome 9p21 loss.^{8,50} By examining somatic mutations of a large ccRCC next-generation sequencing cohort and comparing first-line sunitinib versus first-line everolimus, we demonstrated that patients with *PBRM1* mutation benefited significantly from first-line sunitinib and first-line everolimus with first-line progression-free survival (PFS1L) times of 11.0 and 12.8 months, respectively.⁸ However, patients with *BAP1* mutations derived a less pronounced benefit from first-line sunitinib (PFS1L, 8.1 months) and marginal to no benefit from first-line everolimus (PFS1L, 4.9 months).⁸ In the metastatic ccRCC setting, *PBRM1* mutation is a stronger prognostic marker associated with favorable responses to most of the currently approved treatments including immune checkpoint inhibitors. However, *BAP1* mutations are probably responsive to anti-VEGFR tyrosine kinase inhibitors and irresponsive to mTORC1 inhibitors. Besides *PBRM1* and *BAP1*, somatic mutations in *SETD2*, *KDM5C*, *TSC*, *PTEN*, *MTOR*, *TP53*, and *TERT* have also shown early promise in prognostication.⁶⁷⁻⁷⁰

With the improvement in histopathologic diagnosis, advances in multiomics-based molecular pathologic classification, and emergence of biomarker-centered functional pathology, the oncology community is at the early stage of implementing precision-based cancer care. Hence, we envision that an applied pathology approach integrating mechanisms, animal models, multiomics, clinical trials, and biomarkers could be of value in the future advancement of personalized, precision cancer care.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Support

Supported by National Institutes of Health (NIH) Grant No. R01 CA223231 (J.J.H., V.H.L., T.O., and E.H.C.), a National Cancer Institute intramural grant (C.J.R.), and NIH Grant No. R01 CA224917 (T.H.H.).

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**Chromosome 3p Loss—Orchestrated VHL, HIF, and Epigenetic Deregulation in Clear Cell Renal Cell Carcinoma**

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Consulting or Advisory Role: Exelixis, Genentech, Pfizer, Ipsen

Research Funding: Novartis

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