Review

Genomics and genetics of clear cell renal cell carcinoma: a mini-review

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Abstract

Renal cell carcinoma (RCC) represents a heterogeneous group of malignancies derived from the kidney, of which clear cell RCC (ccRCC) accounts for nearly 75% of cases. Despite major advances in effective therapies, metastatic ccRCC is still associated with a 10%-20% 5-year survival and remains quite lethal. Great effort has been placed into understanding the genetics and genomics of ccRCC and their prognostic and therapeutic implications. Large-scale cancer genomics sequencing studies have identified several driver genes beyond VHL, particularly PBRM1 (40%), SETD2 (15%), and BAP1 (10%), drastically changing the concept of single-gene pathology underlying sporadic ccRCC. In this mini-review, we explore the pathways by which the loss of VHL, PBRM1, SETD2, and/or BAP1 induce ccRCC through discussion of gene function, disease models, prognostic indications, and therapeutic advances.

Keywords: Kidney cancer, oncogene, tumor suppressor gene, VHL, HIF, PBRM1, BAP1, MTOR

INTRODUCTION

Renal cell carcinoma (RCC) represents a heterogeneous group of malignancies derived from the kidney, of which clear cell RCC (ccRCC) accounts for nearly 75% of cases. ccRCC is characterized by lipid- and glycogen-rich cytoplasm, which appears clear following histologic processing. ccRCC has generally been defined by biallelic loss of the VHL tumor suppressor gene, located on 3p25 - loss of heterozygosity of 3p has been demonstrated in > 90% of ccRCC cases and complete loss of the VHL gene via genetic and/or epigenetic mechanisms have been shown in > 80% of cases. Loss of VHL leads to uncontrolled activity of hypoxia-inducible transcription factors (HIFs), which promotes the inhibition of mitochondrion and redirection of glucose and glutamine for glycogen and lipid synthesis, leading to the classic morphological appearance of ccRCC.
However, due to large-scale cancer genomics sequencing efforts, several driver genes beyond VHL have been identified, and the concept of single-gene pathology underlying sporadic ccRCC has drastically evolved\(^4\). Prevalent gene mutations identified by these consortium studies included PBRM1 (40%), SETD2 (15%), and BAP1 (10%). These tumor suppressor genes are all located on 3p21 [Figure 1] and encode for chromatin and/or histone modifiers, suggesting epigenetic dysregulation as a convergent pathogenic signature in ccRCC\(^{5,6}\). Given the close genomic localization of all 4 genes spanning chromosome 3p21-25, the allelic loss of 3p is a fundamental component of ccRCC pathogenesis [Figure 1]\(^2\). These pathways by which the loss of VHL, PBRM1, SETD2, and/or BAP1 induce disease will further be discussed in relation to function, disease models, prognostic indications, and therapeutic advances.

**THE VHL/HIF PATHWAY**

**VHL**

*VHL* plays a key role in the pathogenesis of ccRCC and its inactivation has been identified as the earliest and fundamental driving event in the development of ccRCC. The *VHL* tumor suppressor gene encodes for VHL, a component of the E3 ligase complex responsible for ubiquitination of hypoxia-inducible transcription factors 1α and 2α (HIF-1α and HIF-2α) for proteasome-mediated degradation\(^7,8\). In a normal oxygen state, HIF-1α and HIF-2α are modified by prolyl hydroxylase domain (PHD) proteins that allow VHL recognition and binding, leading to the rapid degradation of these HIF proteins\(^7,9\). Hypoxic conditions inactivate PHD proteins that perform the posttranslational prolyl hydroxylation of HIFs for degradation, thereby stabilizing HIF-1α and HIF-2α\(^7\). Similarly, loss of VHL function by somatic mutation, hypermethylation, or other genomic alterations of *VHL*, results in loss of HIF protein degradation and uninhibited HIF activity regardless of oxygen status. Recent genomic studies have also identified a group of ccRCC tumors that lack the characteristic 3p loss and *VHL* mutations but also display overexpression of HIF proteins\(^10\). These tumors are characterized by hotspot mutations in transcription elongation factor B (*TCEB1*), which encodes for protein elongin C, a subunit of the Elongin (SIII) complex that plays a critical role in eukaryotic transcription elongation\(^11\). This complex is crucial for the recruitment of VHL to the VCB (VHL-elongin C-elongin B) E3 ubiquitin ligase complex that is responsible for HIF degradation, leading to the unregulated HIF activity. Though *TCEB1*-mutated RCC tumors have yet to be established as a distinct subtype, identification of loss of *TCEB1* and its effect on HIF activity supports the central HIF-driven pathogenic theme in ccRCC tumors. Furthermore, the activation of the HIF signaling pathway is also observed in several additional RCC subtypes, such as clear cell papillary RCC tumors which are morphologically and clinically distinct from ccRCC\(^12\).

**HIF**

Upon stabilization, HIF-1α accumulates and directly regulates cellular metabolism in coping with the low oxygen environmental stress. Additionally, HIF-1α, like HIF-2α, dimerizes with HIF-1β or aryl hydrocarbon receptor nuclear translocator, producing an active transcription factor. HIF-1 and HIF-2 dimer complexes then activate and upregulate the transcription of numerous hypoxia-inducible targets genes, many of which mediate cell metabolism and growth\(^7\). These target genes differ among different cell lines and tissues, and the sets of genes regulated by HIF-1α and HIF-2α, respectively, overlap but are not identical\(^13\). In kidney cells, glycolysis is primarily driven by HIF-1α, whereas HIF-2α regulates expression of vascular endothelial growth factor A (VEGFA), cyclin D1, erythropoietin, and C-X-C chemokine receptor 4 \(^14\). Increased HIF-1α levels have been associated with increased mortality in many types of human cancers\(^15\). However, the role of HIF-1 and HIF-2 in ccRCC has not yet been fully elucidated, as they seem to both affect the development and progression of ccRCC. Mouse models with constitutive HIF-1α, not HIF-2α, expression in the renal proximal tubule developed ccRCC\(^16,17\). Elimination of HIF-2α in VHL-/- ccRCC xenograft assays can suppress their ability to form tumors in nude mouse, while HIF-2α overproduction can override intact VHL tumor suppressor function\(^14,18,19\). Furthermore, HIF-2α single nucleotide polymorphisms have
been associated with increased risk of developing kidney cancer in the general population\textsuperscript{20}. Therefore in human ccRCC, disease progression and mortality have been associated with HIF-2α overexpression rather than HIF-1α, which is often silenced by gene deletion\textsuperscript{21}. Altogether, HIF-1 probably plays an essential role in tumor initiation, whereas HIF-2 is needed for tumor progression.

Identification of HIFs as renal oncogenes and their downstream targets have led to development of many currently approved and investigational therapies for systemic treatment of advanced or locally unresectable ccRCC. Given the dependence of ccRCC tumors on angiogenesis for growth, inhibitors of VEGF and VEGF receptor tyrosine kinases, such as bevacizumab, sorafenib sunitinib, pazopanib, and axitinib, cabozantinib, and lenvatinib have been developed and are widely used as first or second-line therapies [Figure 2]\textsuperscript{22}. Small
molecule inhibitors of HIF-2α have also been developed\textsuperscript{[23]}\ldots HIF-2α antagonism may theoretically provide additional efficacy than VEGF inhibitors\textsuperscript{[24]}. Currently under investigation, PT2385, a direct HIF-2α inhibitor, has shown favorable safety profile and activity in patients with previously treated advanced ccRCC\textsuperscript{[9]}. Additional approved and investigational treatments for ccRCC will be discussed in the “Current Therapies” section.

**EPIGENETIC AND CHROMATIN REGULATION PATHWAYS IN CCRCC**

Though it plays a key role in the pathogenesis of ccRCC, VHL loss alone is not sufficient for ccRCC development. Given the latency of ccRCC formation in human VHL syndrome and the inability to induce ccRCC in \textit{VHL}-deficient mice\textsuperscript{[25]}, ccRCC development requires additional genetic and/or epigenetic events to occur. In order to characterize these driving events, several prevalent novel gene mutations have been identified, including \textit{PBRM1}, \textit{SETD2}, and \textit{BAP1}. Located on 3p21, these genes encode for tumor suppressor chromatin- and histone-modifying proteins\textsuperscript{[26]}, and unlike \textit{VHL} loss, \textit{PBRM1}\textsuperscript{[27]}, \textit{SETD2}\textsuperscript{[27]}, and \textit{BAP1}\textsuperscript{[27,28]} mutations are associated with more clinical progression of individual stages of ccRCC.

**PBRM1**

\textit{PBRM1} was first identified as the second most commonly mutated gene in ccRCC in 2010\textsuperscript{[29]}. Confirmed in subsequent large-scale genomics studies, \textit{PBRM1} mutations occur in about 40% of human ccRCC cases\textsuperscript{[4,30]}. \textit{PBRM1} encodes for protein BRG1-associated factor (BAF) 180, a critical subunit of the polybromo BAF SWI/SNF chromatin remodeling complex\textsuperscript{[30]}. SWI/SNF chromatin remodeling complexes are large macromolecular structures that mobilize nucleosome via ATP consumption, thereby modulating chromatin structure to regulate vital cellular processes, including cell cycle, cell fate, cell death, metabolism, and DNA repair\textsuperscript{[10-12]}. Detected in about 20% of human cancers\textsuperscript{[25]}, mutations in SWI/SNF proteins and other epigenetic regulators encompass a major class of cancer genes, demonstrating preferential enrichment by cancer type\textsuperscript{[34]}. \textit{PBRM1}, highly mutated in ccRCC, has been shown to play a critical role in the tumorigenesis of ccRCC.

Several studies have established the tumor suppressor role of \textit{PBRM1} via analysis of pre-neoplastic renal cells in kidney-specific \textit{PBRM1}- and \textit{VHL}-deficient mouse models and in RCC cell lines \textit{A704} and \textit{786-O} \textit{in vitro}\textsuperscript{[30,35]}. Mechanistically, genetically engineered mouse kidney cancer models demonstrated that \textit{PBRM1} functions to prevent kidney tumor cell growth by restraining the self-propagating over-amplification of HIF1 signaling by limiting the HIF1-STAT3 feed-forward loop\textsuperscript{[30,35]}. In the same \textit{PBRM1}--/-- \textit{VHL}--/-- mouse model study, a long latency period of > 6 months was observed, again suggesting the involvement of an additional oncogenic event\textsuperscript{[26]}. Activation of the mechanistic target of rapamycin complex 1 (mTORC1) pathway as the preferred third driver event was observed in several \textit{in vivo} studies\textsuperscript{[16,30]} as well as in human ccRCC cases carrying \textit{VHL} and \textit{PBRM1} mutations\textsuperscript{[16]}. A key cellular complex, mTORC1 integrates nutrient and growth factor signaling to promote anabolic metabolism, supporting tumor growth and invasion\textsuperscript{[36]}. REDD1, a transcriptional target of HIF1, suppresses mTORC1 activity via activation of TSC1/TSC2, functioning as a tumor suppressor checkpoint that limits the oncogenic potential of HIF1\textsuperscript{[37]}. Thus, the activation of the mTORC1 pathway and abrogation of the intrinsic tumor suppressing activity of REDD1 and TSC1/2 may act as the final step to ccRCC development.

Ample clinical evidence support the importance of mTORC1 activation in the pathobiology of human ccRCC\textsuperscript{[18]}, and mTOR inhibitors provide known therapeutic benefit in the treatment of metastatic ccRCC\textsuperscript{[16]}. Correspondingly, \textit{PBRM1} mutation has been associated with longer progression-free survival with everolimus, an mTOR inhibitor, vs. wild-type \textit{PBRM1} (12.8 months vs. 5.5 months)\textsuperscript{[40,41]}. Similarly, patients carrying truncal mutations in the mTORC1 signaling pathway, including \textit{TSC1}, \textit{TSC2}, and \textit{mTOR}, also benefited from mTOR inhibition\textsuperscript{[41,43]}. Therefore, both clinical and preclinical data support the importance of loss of \textit{PBRM1} as the preferred second event and the activation of mTORC1 as the preferred second/third driver event in ccRCC tumorigenesis following VHL inactivation.
In particular to integrate mechanistic and therapeutic research completed on these pathways, we propose a model depicting the interconnection of VHL-HIF-PBRM1-TSC-mTORC1 in the development in ccRCC. This model involves three inactivating steps: (1) VHL loss; (2) VHL-PBRM1 loss; (3) VHL-PBRM1-TSC loss. The initial complete pathologic loss of VHL via chromosome 3p loss, mutations, and/or promoter methylation results in pseudohypoxia - a hypoxia-like molecular response despite normoxic conditions due to aberrant accumulation of HIF proteins. As observed in both human and mouse VHL loss models, the loss of VHL is insufficient in initiating ccRCC development. The subsequent loss of PBRM1 signifies the second step HIF-PBRM1-STAT, which leads to a dysregulated feed-forward amplification loop of maximal downstream gene expression. As evidenced by the PBRM1/-/- VHL/-/- mouse model, the activation of mTORC1 either by loss of TSC suppression or by aberrant increase in mTORC1 activity may encompass the third oncogenic step in ccRCC.

SETD2

SETD2 mutations are observed in 10% of human ccRCC primary tumors, and the frequency dramatically increase to ~30% in metastatic ccRCC patient samples, thereby representing an important molecular aberration in ccRCC metastatic progression. SETD2 encodes a histone H3 lysine 36 methyltransferases that utilize a conserved SET domain. Most significantly, SETD2-gene products are responsible for the trimethylation of the histone H3K36, loss of which is associated with widespread RNA processing defects, thereby affecting chromatin accessibility, transcriptional activation, DNA repair, and cell cycle regulation. SETD2 functions as a tumor suppressor in several types of cancer, including breast cancer and leukemia, and post-transcriptional regulation of SETD2 may be correlated with tumor development in breast cancer and ccRCC; additionally, several studies suggest inactivating mutations of SETD2 occur most prevalently in ccRCC and non-small cell lung cancer.

SETD2 mutations are clinically associated with worse kidney cancer specific survival and ccRCC metastasis, though SETD2 loss is not correlated with poor targeted treatment outcomes. Tumor associated SETD2 mutations also highlight the presence of significant intratumor heterogeneity in ccRCC. Multiple SETD2 mutation variants have been identified among different tumor regions in individual patients, suggesting its role in the convergent evolution of ccRCC. Given its frequency of inactivation in ccRCC and its critical role as a tumor suppressor, loss of SETD2 function represents an important genomic progression marker in ccRCC.

BAP1

BAP1, like SETD2 and PBRM1, is found on chromosome 3p21. BAP1 encodes for a deubiquitinating enzyme, shown to bind breast cancer type 1 susceptibility protein (BRCA1) and BRCA1-associated RING domain protein 1 (BARD1) and thereby function as a tumor suppressor by inhibiting the ability of BRCA1/BARD1 to mediate ubiquitination and autoubiquitination. BAP1 also regulates transcription via histone modulation, thus playing a key role in cell cycle and growth, cellular response to DNA damage, and chromatin dynamics. BAP1 mutations are prevalent in about 10% of human ccRCC cases, and loss of BAP1 function is associated with tumors of high grade and large size as well as poor overall clinical outcome despite targeted therapy. Kidney-specific homozygous deletion of VHL and BAP1 in mice resulted in early death, though some mice with heterozygous deletion of BAP1 and complete loss of VHL developed tumors only moderately resembling human ccRCC. Further preclinical investigation of the BAP1 interaction with VHL inactivation is required to more clearly define the role of BAP1 in ccRCC tumorigenesis.

CURRENT AND EMERGING THERAPEUTICS

The management of metastatic ccRCC has improved dramatically over the last decade. Prior to 2005, only two drugs were approved for the medical treatment of advanced ccRCC and the median survival was poor (15 months). The introduction of targeted therapies expanded the treatment options greatly and
doubled the median survival (~30 months). Given the highly vascular nature of ccRCC, tyrosine kinase inhibitors targeting the VEGF signaling pathway provided considerable benefit over the interleukin-2 (IL-2) and interferon treatments. Seven anti-angiogenic drugs have been approved for first-line and second-line treatment of metastatic RCC since 2005, including sorafenib, sunitinib, pazopanib, axitinib, bevacizumab, cabozantinib, and lenvatinib. With the exception of lenvatinib in combination with everolimus and bevacizumab use with interferon-α, all approvals for VEGF-targeted therapies have been for single agents. The development of mTOR inhibitors, temsirolimus and everolimus, have provided additional therapeutic benefit as second-line single agents or in the first-line setting in patients with poor risk status. Despite these therapeutic advances, the average duration of disease control with these drugs remains only 8-9 months for first line treatment and 5-6 months in the second line setting. Additional target therapies under investigation also include PT2385, a first-in-class HIF-2α antagonist, which has shown a favorable safety profile and activity in a phase I dose escalation trial.

The new generation of immunotherapies has also shown promise for the treatment of metastatic ccRCC. Immunotherapy has long been utilized to treat RCC with the use of interferon-α and high-dose IL-2, but these cytokine therapies were associated with significant toxicity and low response rates. Thus, the development of T-cell immune checkpoint inhibitors may similarly exploit intrinsic antitumor immune activity with fewer adverse effects and improved quality of life. Nivolumab, a monoclonal antibody against programmed cell death protein 1, was approved following the CheckMate 025 randomized control trial, which demonstrated an overall survival benefit of nivolumab over everolimus in patients who failed prior sunitinib or pazopanib therapy. The results of CheckMate 214, a randomized clinical trial investigating nivolumab in combination with ipilimumab, an inhibitor of checkpoint cytotoxic T lymphocyte-associated protein 4 (CTLA4), vs. sunitinib in patients with previously untreated advanced ccRCC, were recently reported. Overall survival and objective response rates of nivolumab plus ipilimumab were significantly higher than sunitinib among intermediate- and poor-risk patients. Thus, this combination has been granted by the FDA as a first-line therapy. Phase III clinical trials of additional combinations of checkpoint inhibitors with VEGF-targeted therapies are under investigation, which probably will again revolutionize the way we treat metastatic kidney cancer.

CONCLUSION
The fields of kidney cancer biology and therapy have undergone transformative changes over the past two decades. However, we are just at the beginning of contemplating how to best integrate these two seems distinct yet highly inter-informative disciplines. The exceptional clinical benefits derived from modern cancer therapy including targeted and immunotherapies have been associated with an astronomical socioeconomic burden to the society. In the authors’ opinion, the principal way for the medical society to further improve treatment efficacy and reduce the cost is the thoroughly integrate multi-omics based predictive biomarkers into standard practice for the selection of the most effective front-line/second line therapeutics with a curative intent. It is a tall but achievable order thus challenge awaiting to be conquered, exemplified are the shown transition of therapeutic eras in metastatic kidney cancer from the “Dark Age” (~2005, 2 drugs, median survival at ~15 moths) through the “Modern Age” (~2015, 9 drugs, median survival at ~30 months) to the “Golden Age” (2015~, 13 drugs and counting, 30 months and counting with significant number of patients expected to be cured). Nevertheless, much needs to be done if we wish to reach Diamond Age by 2025 when most kidney cancer patients could be rendered free of recurrence, progression, and thereby mortality from RCC.
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