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Review

Genomic Instability in Kidney Cancer: Etiologies and Treatment Opportunities

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Abstract. Genomic instability is a hallmark of cancer, allowing for cancer initiation, proliferation, and progression through the accumulation of driver mutations. This instability seen in cancer arises due to a variety of factors in the cancer cell itself as well as in the cell's environment, including endogenous and exogenous stressors leading to DNA damage in the setting of deficiency in DNA damage response (DDR). While genomic instability is beneficial to cancer cell growth and survival, it also creates targetable vulnerabilities in the cell. Kidney cancer displays low to moderate genomic instability, yet does not have frequent mutations in canonical DDR genes and is not typically responsive to DNA damaging therapies. In this review, the etiology of genomic instability in kidney cancer, with a primary focus on clear cell renal cell carcinoma (ccRCC) histology, is discussed; and, pre-clinical data supporting the use of agents targeting DDR in ccRCC is summarized with associated progress towards clinical applications.

INTRODUCTION

Genomic instability is a driving force behind cancer initiation and progression [1, 2]. In general, this instability arises due to increased stress on the cell from endogenous and exogenous sources combined with defects in DNA repair mechanisms. Mutations in the genome accumulate, which may result in insurmountable damage and cell death, or can result in the cell acquiring functional mutations in cancer-driving genes that allow for continued cell survival and proliferation despite this instability. All cancers display some degree of genomic instability; however, large scale DNA sequencing studies have revealed that genomic instability, as measured by the tumor's mutational burden (TMB) and/or copy number variation (CNV), is variable across cancer tissue type and is also variable even within a single cancer type due to differing genotypes [3, 4].

Identifying defects in DNA damage response (DDR), chromatin organization, and replication stress response (RSR) in a tissue and gene-mutation specific manner can not only inform the underlying etiology of genomic instability in cancer, but also can divulge potential treatment strategies that take advantage of these aberrations [5, 6]. For example, poly(ADP)-ribose polymerase (PARP) inhibitors have been shown to selectively kill cancer cells that are deficient in homologous recombination repair (HRR), the more faithful mechanism of DNA double strand break (DSB) repair [7]. This concept of synthetic lethality has been seen in the clinic as well, whereby patients with cancers associated with deleterious variants in *BRCA1* or *BRCA2* display significant responses to PARP inhibitors [8, 9].

Patients with mismatch repair (MMR) deficiency as well as patients with DNA DSB repair deficiency display increased response to immune checkpoint

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blockade (ICB) therapy [10, 11]. This response is likely multifactorial, including the generation of neoantigens, as is the case in tumors with hypermutated phenotypes, such as in MMR-deficiency; but also, preclinical studies have shown there is likely contribution from innate immune activation in the setting of DDR deficiencies leading to the accumulation of S-phase specific DNA damage [12, 13].

Kidney cancer displays a low to moderate level of genomic instability in pan-cancer genomic analyses [3, 14–16], with clear cell renal cell carcinoma (ccRCC) and papillary RCC showing higher mutational burdens than chromophobe RCC. Interestingly, patients with ccRCC in general display a relatively narrow distribution of TMBs as compared to other tumor types such as melanoma, lung cancer, urothelial cancer, and colorectal cancer where hypermutated phenotypes exist [3]. Despite this moderate level of genomic instability as a whole, RCC is not typically responsive to DNA damaging therapies such as platinums or radiation; and, RCC does not display frequent mutations in canonical DDR genes, such as *BRCA1/2* or MMR genes. In addition, TMB (as indicative of neoantigen load) has not been shown to be predictive of an immune response in kidney cancer as it has in other tumor types [17, 18].

The genomic landscape and driver-gene mutations differ between the different histological subtypes of RCC. The most common genomic event in ccRCC is loss of chromosome 3p, which is seen in almost all cases. The most frequently mutated gene in both germline-associated and sporadic kidney cancer is the *Von Hippel Lindau* (VHL) gene, which resides on chromosome 3p. In addition, other genes that reside on 3p and play key roles in chromatin regulation, including *PBRM1*, *SETD2*, and *BAP1,* are also mutated in certain ccRCC cases and impact prognosis. Non-clear cell RCC histologies do not display mutations in VHL to any significant degree, and in general are driven by metabolic pathway derangements.

Despite recent treatment advances for patients with kidney cancer, advanced stage disease remains lethal, and the field lacks biomarkers to select patients for ICB and guide appropriate combination approaches. In this review, preclinical and clinical data highlighting the engines of instability in kidney cancer, with particular focus on ccRCC, are summarized in the context of genotypic and phenotypic background, and the opportunities for therapeutic strategies and associated biomarker development centering on targeting genomic instability are discussed.

VHL AND ccRCC

The vast majority of ccRCC tumors have biallelic loss of *VHL*, which has been shown to be an early, founding event in renal cell carcinogenesis. Given VHL's role in ccRCC initiation and that ccRCC is not known to harbor frequent mutations in canonical DDR genes, multiple preclinical mechanistic dissections of VHL's role in generating genomic instability have revealed VHL plays a role in mitotic fidelity, replication stress response, and even DSB DNA repair.

VHL is an E3 ubiquitin ligase with the most well-known function being regulation of hypoxia inducible factor (HIF). In normoxia, VHL leads to the ubiquitylation and subsequent proteolysis of HIF, whereas in physiologic hypoxia or in cancers with loss of VHL, HIF persists and activates downstream HIF-dependent growth factors and angiogenesis.

Hypoxia alters cancer cell metabolism and also has been shown to directly and indirectly induce genomic instability and impact the expression of DDR-related genes. Importantly, the response to hypoxia is dynamic, with acute hypoxia having differing effects on DDR compared to chronic hypoxia. Immediately after induction of hypoxia, post-translational modifications of HIF occur in conjunction with modifications in key DDR signaling pathways, including phosphorylation and activation of the ATM/CHK2 [19], ATR/CHK1 [20], and DNA-PK [21] axes. Reoxygenation following acute hypoxia leads to increased DNA damage and can give way to cell cycle arrest and even apoptosis in cells with functional p53 [22]. Prolonged, chronic hypoxia however has been shown to lead to the suppression of certain DDR genes in the DSB DNA repair pathway and MMR pathway; however, the exact mechanism of regulation of DDR genes by hypoxia, with parallel increased angiogenesis, and the directionality of DDR gene expression in response to prolonged hypoxia are not completely described, are not necessarily directly related to HIF, and are likely unique based on a tumor's tissue and genetic background.

The loss of VHL in ccRCC leads to persistent activation of HIF and has been shown to give a pseudo-hypoxic state, with dysregulated angiogenesis. Multiple preclinical studies have shown that the loss of VHL impacts mitotic fidelity and DNA DSB repair, and in particular HRR, through a variety of mechanisms, including both HIF-dependent and independent routes. With regards to hypoxia-related DDR regulation, a preclinical study showed

that *VHL*-deficient cells display reduced HRR capacity and decreased expression of certain HRR and MMR genes, including *BRCA1, RAD51, FANCD2*, and *MLH1*. In this study, 786-O human *VHL*-null RCC line was engineered to overexpress VHL and then both lines subjected to hypoxia [23]. The *VHL–/–* cells displayed lower expression of FANCD2, BRCA1, and RAD51 than the VHL-overexpressing isogenic line with the expression of these genes equalized when the VHL-overexpressing cells were exposed to hypoxia; and, thus, the authors posit this DDR gene downregulation is primarily due to the downstream HIF-related effects of pVHL loss [23].

Conversely, multiple studies have shown potential direct, HIF-independent roles for pVHL in HRR regulation. In another preclinical study, pVHL was shown to associate with the suppressor of cytokine signaling 1 (SOCS1) [24], a gene that is highly expressed and associated with ATM in cells undergoing STAT5-mediated oncogene-induced senescence. In cell line models, SOCS1 promoted the nuclear redistribution and K63-ubiquitylation of VHL in response to DNA DSBs, and cells with *VHL–/–* or with *VHL* mutations that compromise its K63-ubiquitylation site display attenuated HRR with persistent DSBs, importantly independent of HIF activity [25]. In a pilot study on the role of VHL loss on DDR signaling, patient tumor data suggests that biallelic loss of VHL leads to deficient ATM pathway activation, loss of p53 protein expression, and increased NHEJ-related protein expression in early ccRCC tumors. In addition, preclinical cell line models of biallelic *VHL* loss show reduced HRR efficacy, increased NHEJ activity, and loss of phosphorylation events downstream of ATM and ATR activity [26].

In addition to VHL promoting HRR following DNA DSBs, the pVHL protein also localizes to the mitotic spindle in mammalian cells and is critical for maintaining mitotic fidelity. Functional inactivation of VHL leads to reduced Mad2 and provokes spindle misorientation, spindle checkpoint deficiency, and subsequent chromosomal instability- all of which can be restored to normal function with re-expression of pVHL [27]. Subsequently, it was shown that pVHL exerts these mitotic regulatory functions *in vivo* as well, wherein loss of pVHL resulted in spindle misorientation and aneuploidy indicative of mitotic checkpoint impairment [28].

Despite the evidence that VHL loss leads to deficient DNA repair and increased replicative stress in preclinical models, *in vivo* mouse models with VHL biallelic mutation alone do not go on to form tumors in the kidney, which indicates that VHL loss is necessary, but alone insufficient, to cause kidney cancer cell transformation and proliferation [29]. Thus, while VHL loss sets the stage for genomic instability in ccRCC, additional genomic aberrations are needed beyond VHL for kidney cancer cell survival, growth, and metastases.

A recent study investigating the factors needed to overcome replicative-stress-induced senescence showed that VHL loss alone induced significant replicative stress with replication fork instability and collapse [29]. In addition, mouse embryonic fibroblast (MEF) cells engineered to be *VHL–/–* showed RAD51 and RPA32 proteins, which are needed for replication fork stability, were significantly reduced compared to parental MEF cells; and, this loss of RAD51 and RPA32 proteins was not due to DDR gene transcriptional repression in the setting of a pseudohypoxic state [23, 29]. Furthermore, the replication stress and accumulation of DNA damage induced by VHL loss were overcome by concomitant mutation in *PBRM1*, a member of the switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex and the second most commonly mutated gene in ccRCC after *VHL*. In this study, *PBRM1* mutation was shown to bypass replication stress induced by VHL loss through an alternative pathway involving a redistribution of H3K9me3 and altering of chromatin structure, thereby stabilizing replication forks. Cells co-deleted for VHL and PBRM1 regained fitness and display the ability to form ccRCC tumors in mice [29]. This study highlights again that biallelic VHL loss induces deficiencies in DNA damage response, both in DNA repair and replicative stress response pathways, leading to genomic instability that is necessary, but not alone sufficient, for kidney cancer initiation. Thus, subsequent genomic events and pathway perturbations beyond VHL and HIF are needed to give way to cancer cell initiation, proliferation, and spread in the face of the instability induced by VHL loss.

GENES INVOLVED IN CHROMATIN MODIFICATION

Outside of *VHL* and *PBRM1*, the next most commonly mutated genes in ccRCC also reside on chromosome 3p and are involved in chromatin modification, with loss of function mutations promoting instability and renal cell carcinogenesis. These

include *BAP1* (*BRCA-associated protein 1*), which is mutated in 10–15% of ccRCC and largely mutually exclusive with PBRM1 mutations, and *SETD2*, which is mutated in 10–15% of ccRCC as well [30, 31].

BAP1 is involved in chromatin remodeling via its deubiquitinase activity of histone H2A and is a tumor suppressor gene inactivated in a variety of cancers including ccRCC [32]. The BAP1 protein product is phosphorylated and recruited to DNA DSBs where it then in turn is responsible for the recruitment of downstream HRR proteins to damage sites, and mediates rapid poly(ADP-ribose)-dependent recruitment of the polycomb deubiquitylase complex PR-DUB to sites of DNA damage [32, 33].

SETD2 is the main methyltransferase responsible for trimethylation of histone-3 at lysine-36 (H3K36me3). Loss of function mutations in *SETD2* are seen in approximately 5% of ccRCC tumors, with mutations occurring at the time of genomic branched evolution [34, 35]. *SETD2* depletion in ccRCC cells led to reduced nucleosome compaction and chromatin association of the key replication proteins minichromosome maintenance complex component (MCM7) and DNA polymerase δ, hindering replication fork progression [34]. Furthermore, H3K36me3 aids in the selection of HRR over NHEJ following DNA DSBs; thus, loss of *SETD2* results in loss of H3K36me3, reduced HRR with ineffective RAD51 loading, increased DNA damage, with increased probability of site-specific chromosome breaks compared to *SETD2* wildtype cells [34, 36].

METABOLIC GENES

In addition to dysregulated angiogenesis, altered metabolism is another key feature of kidney cancer. Germline alterations in TCA cycle genes, *fumarate hydratase (FH)* and *succinate dehydrogenase (SDH)* predispose to hereditary leiomyomatosis and renal cell cancer (HLRCC), characterized by type II papillary RCC, and SDH-related kidney cancer, characterized by RCC of various histologies, paragangliomas, and pheochromocytomas, respectively [37]. Mutations in these genes result in the accumulation of fumarate and succinate, which stabilize HIF and shift cell metabolism towards aerobic glycolysis. In addition to this metabolic shift, the loss of function of TCA cycle genes leads to subsequent build-up of their associated oncometabolites, which in turn have inhibitory effects on α-ketoglutarate (αKG)–dependent dioxygenases [38, 39]. Preclinical data suggest that pathogenic mutations in *FH* or *SDH* lead to suppression of specific αKG–dependent dioxygenases, KDM4A and KDM4B, and indirectly subsequent deficient DNA DSB repair in the HRR pathway [38]. This HRR deficient (HRD) or BRCAness phenotype can be re-capitulated by overexpression of fumarate or succinate in cells that are wildtype for *FH* and *SDH*. Furthermore, these defects conferred sensitivity to PARP inhibition in preclinical models [38, 39].

Further development of identifying oncometabolites as biomarkers of sensitivity to DNA damaging and DDR-directed therapies are ongoing. HIF-activation and altered metabolism are hallmarks of most kidney cancer; yet, despite this reported BRCAness-phenotype, patients with kidney cancer do not typically respond to radiation or DNA damaging chemotherapies, and there are no approved uses of single-agent PARP inhibitors for treating kidney cancer of any histologies. Thus, further research is warranted into the dynamic nature of DNA DSB repair, DDR signaling, and replication stress as kidney cancer progresses, so that these reported DDR deficiencies in kidney cancer can be most-effectively therapeutically targeted.

PI3K/MTOR/AKT PATHWAY ALTERATIONS

The PI3K/mTOR/AKT pathway is a complex web of inter-connected kinases that serve many roles including cell cycle regulation and metabolism, with over-activation promoting cancer cell survival and growth across cancer types. While mutation events in this pathway are relatively sparse in RCC, ccRCC evolution and progression converges on PI3K/mTOR/AKT activation. Mutations in twenty PI3K/AKT-related pathway genes occur in ∼27% of ccRCC tumors, but there is also significant dysregulation of the PI3K/AKT pathway at epigenetic, posttranscriptional, and posttranslational levels in ccRCC [40]. In addition, loss-of-function mutations in *PTEN* and *tuberous sclerosis complex 1/2* (*TSC1/2*) result in de-repression of mTOR signaling, and patients who carry germline mutations in *PTEN* or *TSC1/2* can display a variety of kidney tumor types.

The mTOR/PI3K/AKT pathway has been shown to intersect with DDR signaling at various points in the cascade in multiple preclinical studies. mTOR pathway signaling can in fact drive DDR gene expression, rescuing repair in order to prevent overwhelming

genomic instability in DDR deficient cancer cells and creating resistance to DNA damaging therapies and PARP inhibitors. This highlights an important point in the context of RCC: the status of DDR efficiency versus deficiency and which specific DDR pathways are being utilized are not static in the cancer cell as cancer progresses, and thus tumor and germline mutational information alone is insufficient to capture these dynamic aspects of DDR signaling. Expression-level and functional biomarkers of genomic instability and DDR activity are needed to better understand the underlying biology as well as guide therapy for patients with kidney cancer.

THERAPEUTIC TARGETS OF DDR IN KIDNEY CANCER

The discovery that PARP inhibition selectively kills BRCA1 and BRCA2-deficient cancers serves as the prototype of modern synthetic lethal treatment strategies. Clinically approved uses for PARP inhibitors currently are for select patients with ovarian or breast cancer with biomarkers centering on identifying HRD. However, many studies are ongoing for the use of PARP inhibition in other tumor types and biomarkers, both as single-agent and in combination approaches [6, 7]. As previously mentioned, kidney cancer on the whole lacks mutations in canonical HRR genes; however, there is preclinical evidence, and clinical trials in development, for the use of PARP inhibition in select ccRCC models (Table 1).

As previously mentioned, loss of VHL has been shown to impact HRR activity following DNA DSB. A recent study showed that VHL–/– RCC cells rely heavily on aspartate from reductive carboxylation of αKG to maintain de novo pyrimidine biosynthesis (41). Furthermore, a glutaminase-1 inhibitor induced nucleoside depletion, generated reactive oxygen species, and enhanced replication stress leading to suppressed cell growth in VHL–/– RCC cells. The combination of the glutaminase inhibitor with the PARP inhibitor, olaparib, let to synergistic efficacy, specific to VHL–/– cells [41].

Other driver mutations in RCC also have been shown to lead to PARP inhibitor sensitivity by way of creating an HRD phenotype. For example, preclinical studies have shown a synthetic lethal relationship between PARP1 and BAP1 with BAP1-deficient cells showing sensitivity to PARP inhibition [32, 42], thus clinical trials testing PARP inhibition in BAP1-mutant tumors, including mesothelioma [43] and RCC amongst others, are in development (Table 1). Loss of function mutations in *ARID1A* occur across a variety of cancers, including ccRCC, and mutations in this gene have been shown to give sensitivity to both PARP [44] inhibition and ATR inhibition [45] in preclinical cancer models, and PARP-inhibitor based clinical trials that select for patients with *ARID1A* mutations, including patients with ccRCC, are ongoing (e.g. NCT02576444).

Mutations in *SETD2* leading to H3K36me3-deficiency and contributing to genomic instability also result in a synthetic lethal interaction with WEE1 blockade due to depletion of dNTP pools in the cancer cell [46]. There is a WEE1 inhibitor under clinical development (AZD1775), with a single-agent trial for patients with SETD2-deficient solid tumors, including ccRCC (Table 1).

Cancer cells that generate oncometabolites as a byproduct of TCA cycle gene mutations also lead to an HRD phenotype as previously discussed and engender single-agent PARP inhibitor sensitivity in preclinical models [38, 39]. These mutations and oncometabolites are also under development

Table 1

Gene	DDR[1] Pathway Impacted by Loss of Gene Function	DDR Inhibitor Clinical Trials in Kidney
VHL	HRD[2] through multiple mechanisms, replication stress increased, replication fork stalling, reduced mitotic fidelity	PARP inhibition + Glutaminase Inhibitor (e.g. NCT03875313, talazoparib+telaglenastat)
PBRM1	Replication fork stabilization, altered chromatin structure	NTD
SETD2	Loss of H3K36me3 selection of HRR[3] over NHEJ[4], subsequent HRD, dNTP pool depletion	WEE1 inhibition (e.g. NCT03284385, adavosertib)
BAP1	Loss of recruitment of downstream HRR proteins resulting in HRD.	(e.g. NCT03207347, niraparib; NCT03786796, olaparib)

[1]DDR = DNA damage response; [2]HRD = homologous recombination repair deficiency; [3]HRR = homologous recombination repair; [4]NHEJ = non homologous end joining repair.

as biomarkers for selecting patients for PARP inhibitor treatment, with clinical trials using the PARP inhibitor, olaparib, for *IDH1/2*-mutant brain tumors for example (NCT03561870).

There is mounting preclinical evidence, and clinical trial data, supporting the combination of PARP-inhibitors with targeted therapies against oncogenes that drive HRR gene expression, thereby inducing an HRD phenotype and extending the benefit of PARP inhibition beyond BRCA-mutant carriers. Both mTOR/PI3K pathway inhibitors and anti-angiogenic tyrosine kinase inhibitors- both classes of drugs which have approved uses in kidney cancer- have shown synergy when combined with PARP inhibitors in preclinical models and in clinical trials, even in patients who do not harbor mutations in HRR genes [47–51]. It has not yet been investigated if mTOR/PI3K or angiogenic signaling is driving HRR gene expression in ccRCC models; however, further RCC-specific studies into these combinations are warranted.

Lastly, DDR pathways and immune signaling and activation have significant crosstalk, whereby deficiencies in certain DDR pathways can lead to immune activation. Mismatch repair deficiency is the prime example of neoantigen driven response to immune checkpoint therapy [10]. In addition, defects in DNA DSB repair pathways, including HRR, predict for response to immune therapy for likely multifactorial reasons including neoantigens, but also build-up of cytosolic DNA and activation of innate immune signaling [12, 52, 53]. The preclinical rationale and early clinical data for the combination of DDR inhibitors, such as PARP inhibitors, with ICB is rapidly expanding, with proven safety and early signs of efficacy across various tumor types including breast and prostate [54, 55]. Currently, the majority of patients with metastatic kidney cancer will not obtain a durable, objective clinical response to single-agent immune checkpoint therapy; and, there may be a role for biomarker-driven combinations of DDR inhibitors with ICB for patients with kidney cancer pending further preclinical investigation.

CONCLUSIONS

RCC displays a moderate level of genomic instability with a relatively tight distribution of TMBs across individual patient tumors [14, 15], yet does not respond to traditional DNA damaging chemotherapies. Recent large scale genomic and expression level profiling of ccRCC combined with mounting preclinical dissection of mechanisms underlying RCC initiation and progression have revealed that each of the common driver genes found in RCC play a role in DDR and replication stress response. Thus, even though RCC does not display mutations in canonical DDR genes, such as *BRCA1* and *BRCA2*, there is evidence for targetable, mutation-specific DDR deficiency in RCC tumors. Further studies of patients with RCC that integrate genomic profiling with functional readouts of metabolic, DDR, and immune activity in the tumor and surrounding microenvironment will help guide the application of DDR-inhibitor based therapies for patients with kidney cancer.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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