VHL mutations predict survival of patients with renal cell carcinoma in response to the therapy of immune checkpoint inhibitors

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Abstract

**Purpose:** To identify a predictive biomarker of immune checkpoint inhibitor (ICI) therapies for renal cell carcinoma (RCC).

**Methods:** Survival analysis of mutations in a panel of 468 cancer-related genes in a cohort of 151 RCC patients who underwent the ICI treatment using Cox regression model univariate or multivariate analysis in publicly available datasets.

**Results:** We found that VHL mutations were the only promising independent predictor for overall survival (OS) (HR=0.44, 95% CI=0.25-0.77 and \( P=0.004 \)). More specifically, compared with 26 months of survival in the wildtype patients, metastatic RCC patients carrying truncated VHL mutations had a significantly longer survival time of nearly 70 months (HR=0.45, 95% CI=0.25-0.82 and \( P=0.008 \)) in the presence of the ICI therapy. This survival benefit was also observed in another cohort of 35 patients with clear cell RCC from Dana-Farber Cancer Institute (DFCI): compared with 29 months in the wildtype patients, patients with VHL truncated mutations also had a longer median OS of 33 months (HR=0.59, 95% CI=0.24-1.44 and \( P=0.243 \)). These observed survival benefits were independent of VHL expression and tumor infiltration immune cells in The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA KIRC).

**Conclusion:** VHL mutational inactivation may have an effect on the RCC response to ICI therapy, likely by the upregulation of PD-L1 via attenuating degradation of HIF-1α. To our knowledge, this is the first report of VHL mutations as an independent predictive biomarker for the ICI therapy in RCC, which, once validated by larger clinical trials, may help improve clinical decision-making in individualized treatment of RCC patients.

Introduction

RCC is one of the most common malignancies in the urinary system. Over 400,000 new patients suffer from RCC along with over 175,000 cancer-related deaths worldwide in 2018 [1]. Due to intrinsic resistance to the chemo-radiotherapy, the mainstay therapy for metastatic RCC (mRCC) has been limited to traditional therapies, such as cytokines or VEGF/mTOR targeting therapies. While these therapies have improved outcomes compared to the chemo-radiotherapy, they are still limited by toxicities or drug resistance that usually occur within the first year after the treatment. As a result, the 5-year over survival of mRCC has remained < 8% for the past decades [2~4]. The immune checkpoint inhibitor (ICI) treatment, including anti-bodies targeting programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA4), provide survival benefits only to a minority of mRCC with a durable anti-tumor effect; there is no obvious improvement of progression-free survival (PFS) in unselected mRCC patients, compared to the mTOR inhibitor therapy [3, 5], making it critical to identify sensitive mRCC patients who can benefit from the ICI treatments.

Recently, PD-L1 expression levels, defect mismatch repair (dMMR) or microsatellite instability high (MSI-H), tumor mutation burden (TMB) have positively demonstrated some predictive effects of the ICI.
treatment on various types of cancer \cite{6-8}. However, to the best of our knowledge, there are few reliable predictive biomarkers for the indications of ICI treatment of mRCC, including TMB that has been proven to be incapable of providing potential survival benefits \cite{8}. As for PD-L1, which is expressed in less than 1% of the tumors in 75% of RCC patients, its role in predicting the treatment response is obviously trivial \cite{9}. Hence, it is pivotal to identify alternative predictive biomarkers for the ICI treatment of mRCC. To this end, we analyzed the existing mutation profiles in tumors for the prediction of the ICI treatment outcomes, because mutations in several genes have been demonstrated to predict outcomes of cancer patients effectively \cite{10}. Hence, in the present study, by utilizing the publicly available mutation data on various cancers, we aimed to find mutations that may serve as potential predictors for overall survival (OS) in RCC patients.

Materials And Methods

Data information

We downloaded all the clinical information, mutations and RNA sequencing data of three independent datasets from the publicly available cBioPortal database (https://www.cbioportal.org), which included the Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) dataset (https://www.cbioportal.org/study/summary?id=tmb_mskcc_2018), The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA KIRC Provisional) dataset (https://www.cbioportal.org/study/summary?id=kirc_tcga), and the DFCI (Dana-Farber Cancer Institute) ccRCC dataset (https://www.cbioportal.org/study?id=ccrcc_dfci_2019).

Initial analysis

In the initial analysis of ICI-related gene mutation data, we used the MSK-IMPACT dataset, considering all somatic mutation data that included missense, frame-shift, nonsense, nonstop, splice site, and translation start site changes, except for the synonymous mutations of the target panel containing 468 cancer-related genes \cite{11}. These data were derived from the MSK-IMPACT assay that was applied to all the 151 mRCC patients who received ICI therapies. We first divided the 151 mRCC patients into three groups according to the 468 genes panel tested: patients having non-mutated genes, patients with certain genes (e.g., \textit{VHL}) with mutations, and patients with other genes with mutations, and then we performed univariate Cox proportional hazards regression analysis for these three groups to evaluate the association between gene mutation status and OS of mRCC patients. We also generated Kaplan-Meier survival curves to visualize the results. Those genes with a \( P \) value <0.05 for a mutation rate >0.05 were considered the candidates for the multivariate Cox regression analysis with adjustment for age, sex, TMB, and ICI regimes. Subsequently, we selected the patients with those genes that had truncated mutations (frameshift insertion, frameshift deletion, nonsense, splice-site) to be compared with those patients with the wildtype genes, and we then performed log-rank test to compare the differences in OS between these two groups of patients.

Replication
To replicate the findings of those ICI-related genes present in mRCC patients included in the above-mentioned patient cohorts, we used another DFCI cohort that had 35 metastatic ccRCC patients who had the similar genes detected for mutations and also received ICI therapies. To compare the survival between the patients carrying the genes with truncated mutations and the patients with wildtype genes, we performed the log-rank test to compare the differences in OS between two groups.

**Further exploration**

To ascertain whether those mutational genes present in the above-mentioned MSK-IMPACT cohort may affect the survival of RCC patients without ICI therapies, we analyzed the TCGA KIRC dataset from 451 ccRCC patients who had somatic mutation data but only received traditional treatments, including surgery, chemo-radiotherapy, cytokines and target therapy. Then, we compared OS among patients carrying all genes with mutations, patients carrying genes with truncated mutations, and patients carrying wildtype genes.

We further evaluated the effect of VHL expression on OS in ccRCC patients by using the TCGA-KIRC dataset by using the median VHL mRNA expression value obtained from RNA sequencing data as the cutoff value to divide the 534 ccRCC patients into two groups of low and high VHL mRNA expression, and we used the log-rank tests to compare the differences in OS between the two groups.

We also compared the differences in tumor-infiltrated immune cells between the groups of the patients carrying VHL mutational genes and patients carrying VHL wildtype genes in the 451 patients from the TCGA KIRC dataset by calculating the gene expression matrix using CIBERSORT online tools (https://cibersort.stanford.edu/) [12].

All the statistical analyses were performed by using R language (version 3.5.1), and P values were two-sided with a significance level of 0.05.

**Results**

The analysis flowchart is shown in Figure 1. In the initial analysis, we used a cohort of 151 metastatic or unresectable RCC patients who underwent the ICI therapy (122 received anti PD-1/PD-L1 monotherapy, and the remaining 29 received a combination of anti CTLA-4 and anti PD-1/PD-L1 therapies). Clinical characteristics of the patients are presented in Table 1. The univariate Cox regression analyses of the three groups (i.e., patients with non-mutated genes, patients with certain genes with mutations, and patients with other genes with mutations) showed that mutations in 21 genes were significantly associated with OS, but the patients with the highest VHL mutation rate (70%) had the largest HR of 1.91, compared with the patients with mutations in other 20 genes (2%) (Supplement Table 1). Therefore, subsequent analyses focused on the VHL mutations.
Table 1
Clinicopathological characteristics of the MSK-IMPACT RCC cohort underwent ICI treatment

| Characteristics                      | No. of Cases (%) |
|--------------------------------------|------------------|
| All subjects                         | 151 (100)        |
| Age at diagnosis                     |                  |
| <30                                  | 3 (2.0)          |
| 31-50                                | 25 (16.6)        |
| 50-60                                | 54 (35.8)        |
| 61-70                                | 51 (33.8)        |
| >71                                  | 18 (11.9)        |
| Sex                                  |                  |
| Female                               | 42 (27.8)        |
| Male                                 | 109 (72.2)       |
| ICI Regime                           |                  |
| PD-1/PD-L1                           | 122 (80.8)       |
| Combo                                | 29 (19.2)        |
| Mean of TMB (/Mb)                    | 4.25             |
| Cell subtype                         |                  |
| Chromophobe Renal Cell Carcinoma    | 5 (3.3)          |
| FH-Deficient Renal Cell Carcinoma   | 2 (1.3)          |
| Papillary Renal Cell Carcinoma      | 5 (3.3)          |
| Renal Cell Carcinoma                | 4 (2.6)          |
| Renal Clear Cell Carcinoma          | 121 (80.1)       |
| Renal Clear Cell Carcinoma with Sarcomatoid Features | 1 (0.7) |
| Renal Mucinous Tubular Spindle Cell Carcinoma | 1 (0.7) |
| Translocation-Associated Renal Cell Carcinoma | 3 (2.0) |
| Unclassified Renal Cell Carcinoma   | 9 (6.0)          |

Abbreviations: MSK-IMPACT, Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets; RCC, renal cell carcinoma; ICI, immune checkpoint inhibitors; Combo, anti CTLA-4 combined with anti PD-1/PD-L1; TMB, tumor mutation burden; /Mb, per Mega bases.
In Figure 2, we present survival curves of patients without mutations in any genes, patients with \textit{VHL} mutations, and patients with mutations in genes other than \textit{VHL}. In the following analysis, we merged the group of patients without mutations or with the \textit{VHL} wildtype and the group of patients with mutations in genes other than \textit{VHL} as one group to be compared with those with \textit{VHL} mutations. In the univariate and multivariate Cox regression analyses for the association between the \textit{VHL} mutation status and OS, we found that the \textit{VHL} mutation status (70.2%, 106/151) was an independent predictor for OS in this patient cohort (HR = 0.52, 95% CI = 0.36-0.77, \(P = 0.001\) and HR = 0.44, 95% CI = 0.25-0.77, \(P = 0.004\) in univariate and multivariate analyses, respectively) (Table 2). Specifically, RCC patients with \textit{VHL} mutations showed a significantly longer OS of 50 months, compared with 26 months in patients with the wildtype \textit{VHL} or mutations in other genes (Figure 3A). The detailed clinical information on the group of patients with \textit{VHL} mutations and the other group is presented in Table 3. It appeared that \textit{VHL} mutations occurred more often in ccRCC (102/121, 84.3%) than in non-ccRCC (n=4/30, 13.3%) (\(P < 0.001\)), and the \textit{VHL} mutation group had a relatively higher mean TMB (4.70±2.46) than that (3.20±3.54) of the \textit{VHL} wildtype group (\(P = 0.003\)). We then focused on truncated mutations, because they were considered functionally important mutations \cite{13}. We found that RCC patients (n = 65) with \textit{VHL} truncated mutations showed a significantly longer median OS of nearly 70 months, compared with 26 months in patients (n = 45) with the wildtype \textit{VHL} (HR = 0.45, 95% CI = 0.25-0.82 and \(P = 0.008\)) (Figure 3B).
### Table 2

Univariate and multivariate analysis of factors associated with overall survival in RCC underwent ICI treatment

| Variables          | Events/Subjects | Univariate analysis | Multivariate analysis |
|-------------------|-----------------|---------------------|-----------------------|
|                   |                 | HR  | 95% CI       | P value | HR  | 95% CI       | P value |
| Age (group in years) | 1.00  | 0.76-1.32 | 0.987 | 1.00  | 0.76-1.32 | 0.994 |
| Sex               | Female (reference) | 21/42 | 1.00 | 1.00 | 1.00 | 1.00 |
|                   | Male | 37/109  | 0.68 | 0.40-1.17 | 0.163 | 0.72 | 0.42-1.25 | 0.294 |
| ICI regime        | Combo (reference) | 8/29  | 1.00 | 1.00 | 1.00 | 1.00 |
|                   | PD-1/PDL-1 | 50/122 | 1.39 | 0.96-2.03 | 0.085 | 1.41 | 0.96-2.06 | 0.077 |
| TMB (/Mb)         | 0.94 | 0.85-1.05 | 0.273 | 0.97 | 0.89-1.06 | 0.532 |
| VHL               | Wildtype (reference) | 24/45 | 1.00 | 1.00 | 1.00 | 1.00 |
|                   | Mutations | 34/106 | 0.52 | 0.36-0.77 | **0.001*** | 0.44 | 0.25-0.77 | **0.004*** |

Abbreviations: RCC, renal cell carcinoma; ICI, immune checkpoint inhibitors. HR, hazards ratio; CI, confidence interval; TMB, tumor mutation burden; /Mb, per Mega bases; Combo, anti CTLA-4 combined with anti PD-1/PD-L1; VHL, von Hippel-Lindau tumor suppressor; * The results were in **bold**, if the P value was less than 0.05.
**Table 3**
Clinicopathological features of patients with a different *VHL* mutation status in the MSK-IMPACT RCC cohort

| Characteristics                        | All patients | Patients with VHL mutations (%) | P value |
|----------------------------------------|--------------|---------------------------------|---------|
| All subjects                           | 151          | 106 (100)                       |         |
| Age (group in years)                   |              |                                 | 0.801   |
| <30                                    | 3            | 0 (0.0)                         |         |
| 31-50                                  | 25           | 18 (68.0)                       |         |
| 50-60                                  | 54           | 38 (70.4)                       |         |
| 61-70                                  | 51           | 38 (74.5)                       |         |
| >71                                    | 18           | 12 (66.7)                       |         |
| Sex                                    |              |                                 | 0.538   |
| Male                                   | 109          | 81 (74.3)                       |         |
| Female                                 | 42           | 25 (59.5)                       |         |
| ICI regime                             |              |                                 | 1.000   |
| PD-1/PDL-1                             | 122          | 85 (69.7)                       |         |
| Combo                                  | 29           | 21 (53.8)                       |         |
| Mean of TMB ± SD                       | 151          | 4.70±2.46 / 3.20±3.54*          | 0.003** |
| Subtype                                |              |                                 | <0.001**|
| Renal clear cell carcinoma             | 121          | 102 (84.3)                      |         |
| Non-clear cell renal cell carcinoma    | 30           | 4 (13.3)                        |         |

Abbreviations: MSK-IMPACT, Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets; RCC, renal cell carcinoma; ICI, immune checkpoint inhibitors; Combo, anti CTLA-4 combined with anti PD-1/PD-L1; TMB, tumor mutation burden; SD, standard deviation. * For those who had the *VHL* wildtype.

**The results were in bold, if the P value was less than 0.05.**

To further ascertain whether *VHL* mutations affected OS in RCC treated with traditional therapies, including surgery, chemo-radiotherapy, cytokines and target therapy, another 451 ccRCC patients from the TCGA KIRC dataset were divided into two groups by *VHL* mutation status with detailed information presented in **Supplemental Table 2**. The *VHL* mutation group (n = 225) showed no survival benefit, compared with the wildtype group (n = 226) (**Figure 3C**). In addition, the *VHL* truncated mutation group (n = 126) also showed no significant survival benefit, compared with the wildtype group in this dataset.
(Figure 3D). Furthermore, there was no association between the tumor infiltrated immune cells and the VHL mutation status in RCC in the TCGA KIRC dataset (Supplemental Table 3).

Additional analysis with the data from the TCGA KIRC dataset suggested that the VHL expression levels did not affect OS in ccRCC patients (n = 534) who did not receive any immunotherapies (Figure 3E), of whom 267 patients had lower VHL expression, while other 267 patients had higher VHL expression.

To replicate the findings in the MSK-IMPACT dataset that the VHL truncated mutation group had a longer median OS than the VHL wildtype group, who all received ICI therapies, we used the data from another DFCI cohort of 35 metastatic ccRCC patients, of whom those (n=16) with VHL truncated mutations also showed a longer median OS (33 months), compared with those (n=10) with the wildtype VHL (29 months), although it did not reach the statistical significance (HR = 0.59, 95% CI = 0.24-1.44 and P = 0.243) (Figure 3F), likely due to a much smaller sample size (n = 35) of this small dataset.

**Discussion**

VHL, as a tumor suppressor, is mutated in over 50% ccRCC that constitutes the majority of RCC. In the present study, the ccRCC accounted for 80.1% RCC in the MSK-IMPACT patient cohort. The VHL gene was mutated in 84.3% and 51% of the ccRCC patients in the MSK-IMPACT cohort and the TCGA KIRC dataset, respectively. Although VHL mutations were significantly associated with TMB in the dataset we used, the TMB was shown to have a little value in predicking the response to ICI treatment. Some studies suggested that the impact of immune cell infiltration on the ICI treatment was not negligible, despite this notion was not well established yet. However, we observed in the present study that the immune cell infiltration rate was not significantly different between tumors with mutational and wildtype VHL.

Truncated mutations have been considered the important functional mutations, usually leading to the loss of gene functions. It has been reported that inactivating mutations of VHL occurred in RCC may attenuate the degradation of HIF, thus leading to the hypoxia signal activation, which stimulates angiogenesis, cell growth and survival of renal cancer cells. In addition, a previous study reported that the hypoxia induced the PD-L1 expression via upregulating HIF-1α expression in tumor-infiltrating myeloid cells, while another study identified that overexpression of PD-L1 and PD-1 was induced in both human in vitro and murine models of hypoxia as well as by HIF-1α transfection. Because mutational inactivation of VHL may attenuate the degradation of HIF, it is reasonable to speculate that the mutational inactivation of VHL could lead to the accumulation of the HIF-1α, thus leading to the PD-L1 up-expression in renal cancer cells, which then makes the cancer cells potentially targetable by the anti-PD-1/PD-L1 therapy. However, more experiments are needed to validate such a hypothesis.

In summary, we found in the present study that VHL mutations, the truncated mutations in particular as an independent predictor for OS, effectively predicted survival of mRCC patients in response to the ICI treatment. Considering other relevant findings from previous studies, we believe that the survival benefit associated with VHL mutations could be due to the upregulation of PD-L1 by VHL mutational inactivation.
via HIF-1α, a possible mechanism that may explain the efficacy of immunotherapies in treating RCC. However, because studies with a limited sample size were used in the present study, additional larger studies or clinical trials as well as functional investigations are needed to confirm these results.

**Declarations**

**Funding**

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**Competing Interests**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Author Contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Zhao LQ, Wu JW and Wu ZH. The first draft of the manuscript was written by Zhao LQ and Qu Q, Zhang J made revisions and approved the final version. All authors read and consented to the final manuscript.

**Data Availability**

All datasets used in this analysis were from the public database, the URLs were presented in the methodology section. The codes used in this analysis can be obtained upon reasonable requests through the corresponding author.

**Ethics approval**

The analysis was a post-hoc study with all the information was de-identified in the data. The Ruijin Hospital Research Ethics Committee has confirmed that no ethical approval is required.

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**Figures**

**Figure 1**

The analysis flowchart. Gene A represent any certain gene of 468 gene panel.
**Figure 2**

The status of \( VHL \) mutations affect the OS of RCC patients.

**Figure 3**

The association of \( VHL \) mutations and expression with OS of RCC patients under ICI therapies or traditional therapies.

**A:** The patients \((n=106)\) with \( VHL \) mutations showed a significantly longer OS than the wildtype population \((n=45)\) in MSK-IMPACT RCC cohort underwent ICI treatment.
B: The patients (n=65) with \textit{VHL} truncated mutations showed a significantly longer OS than the wildtype population (n=45) in MSK-IMPACT RCC cohort underwent ICI treatment.

C: The patients (n=225) with \textit{VHL} mutations showed no significantly benefit than the wildtype population (n=226) in TCGA KIRC dataset underwent traditional therapy.

D: The patients (n=126) with \textit{VHL} truncated mutations showed no significantly benefit than the wildtype population (n=226) in TCGA KIRC dataset underwent traditional therapy.

E: The \textit{VHL} gene expression showed no significantly association with OS of the patients (n=534) in the TCGA KIRC dataset underwent traditional therapy.

F: The patients (n=16) with \textit{VHL} truncated mutations showed a longer OS than the wildtype patients (n=10) in the DFCI patient cohort underwent ICI treatment though the \textit{P} value was not significant due to a smaller sample size.

**Supplementary Files**

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- SupplTables.docx