CLINICAL RESEARCH

Genomic Alterations of Renal Cell Carcinoma and Clinical Implications in the Chinese Population

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Background: The aim of this study was to investigate the genomic alterations of renal cell carcinoma (RCC) in Chinese patients and to evaluate the correlations between significantly mutated genes and tumor mutation burden (TMB) levels in RCC.

Material/Methods: Two batch of specimens were collected from patients with RCC. Cohort 1 enrolled 17 RCC patients. Specimens and clinicopathological data were collected and the duration of disease-free survival were evaluated with a follow-up from 2 weeks to longer than 1 year. Cohort 2 collected 70 clear cell RCC (ccRCC) tissues and blood specimens. Next-generation sequencing were used to detect the genomic variations in those specimens in both cohorts and TMB in cohort 2. Clinicopathological features of the 2 cohorts were collected and the χ^2 test or Fisher's exact test was used for categorical variables stratified by TMB values.

Results: Our present study demonstrated that the top 3 most frequent aberrated genes in Chinese ccRCC patients were ABCB1, UGT1A1, and VHL, with percentages of 50.00%, 42.86%, and 34.52% respectively. And only 1 gene, which was ABCB1, showed statistically significant difference (*P*=0.047) stratified by TMB levels. In addition, 6 oncogenic pathways were involved in ccRCC cases in the 2 cohorts. Only 5 out of the 8 most common altered genes of RCC from COSMIC or TCGA databases were detected in our study.

Conclusions: The genomic alterations of Chinese RCC patients were different from that in TCGA and COSMIC. No significant genomic alterations were found correlating to TMB levels in ccRCC. Non-silent mutation of VHL may be a predictor for the outcome of ccRCC treated with axitinib.

MeSH Keywords: Angiogenesis Inducing Agents • Carcinoma, Renal Cell • Genetic Heterogeneity • Population Groups

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Background

It was estimated that 65 340 new cases of renal cancer will be diagnosed in the USA in 2018, with an estimated 14 970 deaths. Being responsible for over 90% of all cases, renal cell carcinoma (RCC) is the most common type of kidney cancer in adults [1]. The morbidity of renal cancer in male is 2-fold that found in female. The 3 main histological subtypes of RCC are clear cell RCC (ccRCC) which occupies 70% to 80% of cases, papillary RCC (PRCC) which represents 15% to 20% of cases and chromophobe RCC (ChRCC) which represents~5% of cases [2–4]. The morbidity and mortality data of RCC in China was unavailable since RCC was not among the top 10 most common cancers in the Chinese population [5]. A study by Scelo et al. detected the variation in genomic landscape of ccRCC across Europe and suggested that the processes underlying ccRCC tumorigenesis may vary in different populations [2]. The research based on TCGA identified 19 significantly mutated genes in ccRCC, among which *VHL, PBRM1, SETD2, KDM5C, PTEN, BAP1, MTOR,* and *TP53* were most frequently seen [6]. Data of somatic mutations of RCC in Chinese patients was released by a small size study which collected 26 RCC samples. The results of this study showed different frequencies of significantly mutated genes to that of the TCGA, and detected many mutations that were not reported previously [7]. This variation of genomic landscape of RCC in different populations called for research on RCC genomic aberrations in different races.

Tumor mutation burden (TMB), defined as the number of somatic base substitutions and short InDel mutations per megabase (Mb) of genome examined or the total number of somatic missense mutations present in a tumor sample due to different detection techniques, was an emerging biomarker for immune checkpoint inhibitor therapy [8,9]. Kidney cancers possess detectable TMB levels [8]. Cancer patients with high TMB levels have been reported to have better response towards immunotherapy than those with low TMB levels. However, the breakpoint of high TMB levels remains to reach a consensus [10]. Exploring genomic mutations that are strongly correlated with TMB levels may spare the trouble of a breakpoint, thus, is of great significance. To our knowledge, no study has demonstrated the relationship of TMB with significantly mutated genes in RCC.

We carried out the present study to investigate the genomic alterations of RCC in Chinese patients and to demonstrate the correlations between significantly mutated genes and TMB levels in RCC.

Material and Methods

Two batches of specimens were collected from patients with RCC.

Cohort 1, patients and samples

The first cohort (cohort 1) enrolled 17 patients who had undergone surgeries at the Department of Urology at Peking University Third Hospital. Baseline information and clinicopathological data were collected and the duration of disease-free survival (DFS) were evaluated with a follow-up from 2 weeks to longer than 1 year. Blood samples were taken from these patients before surgery, and RCC tissue formalin-fixed and paraffin-embedded (FFPE) specimens were gathered. The pathological subtypes of those RCC samples were confirmed by the pathologists in our hospital.

Written informed consents were obtained from all cohort 1 patients or their consignees. This study was approved by the Ethics Committee of Peking University Third Hospital (Project No. M2017147, Approval No. 2017.126-02).

Cohort 1, DNA extraction and genomic mutations detection

We performed DNA extraction from serial thick sections cut from tumor tissue samples and control sections or blood samples. The invasive tumor content was estimated by pathologists, to ensure more than 50% of cells were tumor cells. The DNA was isolated from the FFPE and blood samples using the DNeasy Blood and Tissue Kit (69504, QIAGEN, Venlo, Netherlands).

The technique of next-generation sequencing (NGS) was carried out to detect the genomic alterations of RCC. We firstly created targeted capture pulldown and exon-wide libraries from native DNA using the 556 NGS panel (Tongshu BioTech, Shanghai, China) and TruePrep DNA Library Prep Kit V2 for Illumina (#TD501, Vazyme, Nanjing, China), and then generated paired-end sequence data using Illumina HiSeq machines.

Cohort 2, patients and samples

Cases in cohort 2 were collected to explore the association between significantly altered genes and TMB. To avoid the bias caused by different pathological subtypes, only ccRCC cases were involved.

In cohort 2, 70 ccRCC tissues and blood specimens, each pair from one patient, were collected from patients who had undergone surgeries at the Department of Urology at Peking University Third Hospital and baseline information was collected, retrospectively. This study was approved by the Ethics Committee of Peking University Third Hospital.

Table 1. The clinical and pathological information of renal cell carcinoma in cohort 1 (n=17).

ID	Age/yr	Gender	Performance status	Histology	Laterality	Surgical approach	TNM stage	DFS/ months
1	54	F	80	ccRCC	L	Lapa	T3aN0M1	0.6
2	76	M	80	ccRCC	L	Lapa	T3bN0M0	14
3	26	M	90	PRCC	L	O	T1aN0M0	9
4	47	M	90	ccRCC	L	Lapa	T3aN0M0	3
5	72	M	100	ccRCC	L	Lapa	T3aN0M0	1
6	68	F	90	ccRCC	R	Lapa	T3aN0M0	2.5
7	50	M	80	ccRCC	R	O	T3aN0M0	2
8	48	M	80	ccRCC	L	Lapa	T2aN0M0	4
9	53	M	90	ccRCC	R	Lapa	T3aN0M0	1
10	49	M	80	ccRCC	L	Lapa	T1bN0M0	3
11	39	M	100	ccRCC	R	Lapa	T1aN0M0	1
12	38	M	80	ChRCC	R	Lapa	T2aN0M0	1
13	61	F	90	ccRCC	L	Lapa	T3N1M0	0.8
14	63	M	90	ccRCC	R	O	T3bN0M0	1
15	68	M	100	PRCC	R	O	T3aN0M0	2
16	72	M	100	ccRCC	L	Lapa	T1bN0M0	1
17	24	M	100	ccRCC	L	Lapa	T1aN0M0	2

F – Female; M – Male; ccRCC – clear cell renal cell carcinoma; PRCC – papillary renal cell carcinoma; ChCC – chromophobe renal cell carcinoma; L – left; R – right; Lapa – laparoscopic nephrectomy; O – open radical nephrectomy.

Cohort 2, DNA extraction and genomic mutations detection

DNA extraction and NGS procedures were the same as that in cohort 1. The technique of NGS was used to define the TMB values in cohort 2 blood samples. Average sequencing depth of coverage was greater than 250×, and more than 99% exons had >100× sequencing depth. TMB was measured in mutations per Mb.

Data analysis

Clinicopathological features of the 2 cohorts were collected and the χ^2 test or Fisher's exact test was used for categorical variables stratified by TMB values. The postoperative DFS duration was assessed. All tests were bilateral, with $P<0.05$ indicating significant statistical difference. Statistical analysis was carried out by the statistical software package SPSS 22.0 (IBM Corp., Somers, NY, USA).

Results

Cohort 1, baseline and clinicopathological information

The baseline and clinicopathological information of patients in cohort 1 are listed in Table 1. As presented, the ages of the 17 patients ranged from 24 to 76 years old, from whom 3 were female patients and the others were male patients. As for the histological subtypes, 14 cases were ccRCC (82.3%), 2 cases were PRCC (11.8%) and only 1 case was ChRCC (5.9%). Four of those patients received open radical nephrectomy among whom 3 had tumors on their right kidneys. The other patients received laparoscopic nephrectomy. The median duration of DFS was 2 months. Three (patient ID 2, ID 14, and ID 15) out of the 17 patients received axitinib, a small molecule anti-angiogenesis drug, and only 1 patient (ID 2) turned out to possess *VHL* aberration.

Cohort 1, Aberrated genes

A total of 22 aberrated genes were found and the top 3 most frequent aberrated genes were *ABCB1, UGT1A1*, and *VHL*.
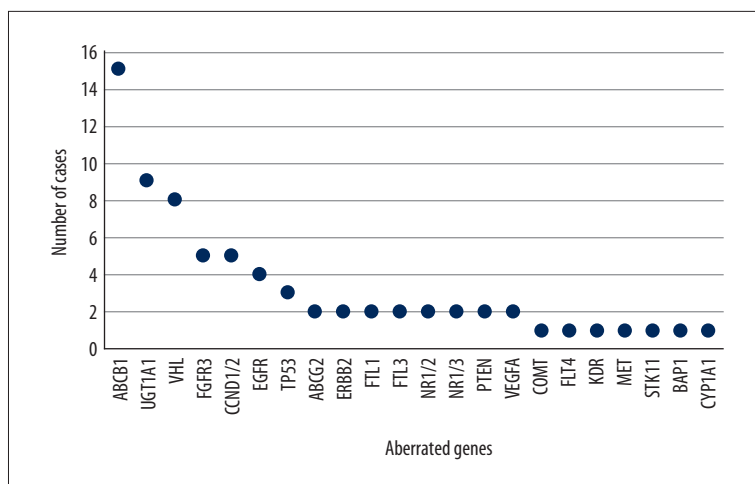
Figure 1. The number of cases for each aberrated gene in cohort 1 (n=17). A total of 22 aberrated genes were found and the top 3 most frequent aberrated genes were *ABCB1*, *UGT1A1*, and *VHL*.

Figure 1 shows the number of cases for each aberrated gene in cohort 1.

Cohort 2, baseline and clinicopathological information

In cohort 2, 70 ccRCC patients were retrospectively involved. The median age was 58.5 years old (range 22 to 83 years). Among the 70 ccRCC patients, 22 were female patients and the others were male patients.

Cohort 2, aberrated genes

The numbers and percentages of genomic aberrations occurred in those 70 ccRCC patients stratified by TMB values are presented in Table 2. A total of 33 aberrated genes were found in cohort 2 and the top 3 most frequent aberrated genes were *ABCB1, UGT1A1*, and *VHL,* in accordance with that in cohort 1.

Cohort 2, TMB

The median value was 3.6 mutations/Mb (range 0 to 22.9 mutations/Mb) in cohort 2. To explore the correlations between significantly mutated genes and TMB levels in ccRCC, we divided the 70 patients into 2 groups according to their TMB level. And the median value (3.6 mutations/Mb) of TMB was chosen as a breakpoint. That was one group (n=34) which presented with higher TMB levels (>3.6 mutations/Mb), and the other group with lower TMB levels (≤3.6 mutations/Mb). As we could see, only 1 gene, which was *ABCB1*, showed statistically significant difference (*P*=0.047) between the 2 groups.

ccRCC, aberrated genes in both cohorts

To evaluate the altered genes further, we summed up the data of ccRCC patients in the 2 cohorts and the percentages of aberrated genes appeared more than once are presented in Figure 2 (n=84). Twenty-one out of 44 aberrated genes appeared

repeatedly when the data of ccRCC patients in 2 cohorts calculated together. The top 3 most frequent aberrated genes in 2 cohorts were *ABCB1, UGT1A1*, and *VHL*, with percentages of 50.00%, 42.86%, and 35.52% respectively.

Pathways involved

Six oncogenic pathways were involved in ccRCC cases (n=84) in the 2 cohorts and the pathways are presented in Figure 3. The altered genes in each pathway were as follows: RTK/RAS pathway (*EGFR, ERBB2, FGFR1, FGFR2, FGFR3, MET, ALK, FLT3, KRAS*) (n=29); PI3K pathway (*PTEN, PIK3CA, STK11, TSC2*) (n=12); p53 pathway (*TP53*) (n=12); cell circle pathway (*CCND1*) (n=9); Wnt pathway (*GSK3B, CTNNB1*) (n=4); and Notch pathway (*FBXW7*) (n=1).

Discussion

Despite its respectively low incidence, RCC possesses a rather high mortality rate due to the delay in diagnosis and lack of effective systemic therapy [1]. The development of targeted anticancer and anti-angiogenesis drugs can improve the outcome for RCC patients [11,12]. As presupposition for those new therapeutic agents, genomic detection turns out to be of essential significance. Besides, genomic detection has a place in prognosis and discovery of drug susceptibility [13,14]. The existing databases of RCC genomic landscape, like TCGA and COSMIC, were mainly based on Caucasian populations. RCC is a highly heterogeneous disease [15]. Therefore, it is important to illustrate the genomic aberrations in Chinese RCC patients. Our present study demonstrated that the top 3 most frequent aberrated genes in Chinese ccRCC patients were *ABCB1, UGT1A1* and *VHL*, with percentages of 50.00%, 42.86%, and 34.52% respectively. And only 1 gene, which was *ABCB1*, showed statistically significant difference (*P*=0.047) stratified by TMB levels. Besides, 6 oncogenic pathways were involved in ccRCC cases in the 2 study cohorts.

Table 2. The percentages of genomic aberrations stratified by TMB values in cohort 2.

| Aberrated genes	Total (n=70)		TMB >Median (n=34)		TMB ≤Median (n=36)		P value
ABCB1	29	(41.43%)	10	(29.41%)	19	(52.78%)	0.047
UGT1A1	27	(38.57%)	10	(29.41%)	17	(47.22%)	0.126
VHL	21	(30%)	10	(29.41%)	11	(30.56%)	0.917
ERBB2	8	(11.43%)	5	(14.7%)	3	(8.33%)	0.402
TP53	8	(11.43%)	4	(11.76%)	4	(11.11%)	0.932
FGFR3	7	(10%)	5	(14.7%)	2	(5.56%)	0.202
MET	6	(8.57%)	4	(11.76%)	2	(5.56%)	0.354
PIK3CA	5	(7.14%)	3	(8.82%)	2	(5.56%)	0.596
CCND1	4	(5.71%)	2	(5.88%)	2	(5.56%)	0.953
CTNNB1	3	(4.29%)	3	(8.82%)	0		0.109
POLE	3	(4.29%)	0		3	(8.33%)	0.240
EGFR	2	(2.86%)	2	(5.88%)	0		0.232
FGFR1	2	(2.86%)	2	(5.88%)	0		0.232
PTEN	2	(2.86%)	2	(5.88%)	0		0.232
TSC2	2	(2.86%)	0		2	(5.56%)	0.493
AKT2	1	(1.43%)	0		1	(2.78%)	1.000
BRAC1	1	(1.43%)	1	(2.78%)	0		0.486
BTG1	1	(1.43%)	1	(2.78%)	0		0.486
CFTR	1	(1.43%)	0		1	(2.78%)	1.000
FGFR2	1	(1.43%)	1	(2.78%)	0		0.486
NSD	1	(1.43%)	0		1	(2.78%)	1.000
NTRK1	1	(1.43%)	0		1	(2.78%)	1.000
PAX5	1	(1.43%)	0		1	(2.78%)	1.000
PRKD1	1	(1.43%)	0		1	(2.78%)	1.000
SETD2	1	(1.43%)	0		1	(2.78%)	1.000
SGK1	1	(1.43%)	0		1	(2.78%)	1.000
GSK3B	1	(1.43%)	0		1	(2.78%)	1.000
STK11	1	(1.43%)	1	(2.78%)	0		0.486
KRAS	1	(1.43%)	0		1	(2.78%)	1.000
ALK EML4	1	(1.43%)	1	(2.78%)	0		0.486
FBXW7	1	(1.43%)	1	(2.78%)	0		0.486
MK-1775	1	(1.43%)	1	(2.78%)	0		0.486
CYP17A1	1	(1.43%)	1	(2.78%)	0		0.486

TMB – tumor mutation burden. The ranges of TMB values were from 0 to 22.9 mutations/Mb and the median value was 3.6 mutations/Mb in cohort 2.
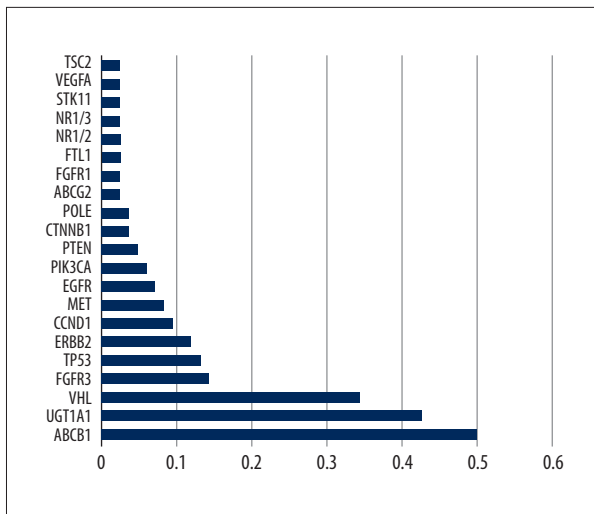
Figure 2. The percentages for aberrated genes appeared more than once in clear cell renal cell carcinoma (ccRCC) patients in 2 cohort groups (n=84). Twenty-one out of 43 aberrated genes appeared repeatedly when the data of ccRCC patients in the 2 cohorts when calculated together. The top 3 most frequent aberrated genes in the 2 cohorts were *ABCB1*, *UGT1A1*, and *VHL*, with percentages of 50.00%, 42.86%, and 35.71% respectively.

Only 5 out of the 8 most common altered genes of RCC from COSMIC or TCGA databases were detected in our study. Compared with COSMIC, those 5 shared genes were *VHL* (34.52%), *SETD2* (1.19%), *BAP1* (1.19%), *CTNNB1* (3.57%), and *TP53* (13.10%) in our present study, while in COSMIC, they were *VHL* (40%), *SETD2* (9%), *BAP1* (9%), *CTNNB1* (7%), and *TP53* (8%). TCGA shared 7 most common altered genes of RCC with COSMIC, the only 1 different gene, which was *CTNNB1* (7%) in COSMIC and PTEN (around 13%) in TCGA. Three genes *PBRM1*, *KDM5C*, and *MTOR* from the 8 did not show significant alteration in our study. To our surprise, the frequencies of altered genes in our study were different from that of another research study based on Chinese RCC patients [7]. The frequency of *VHL* alterations in that study was 67%, which was almost 2 times that found in our study. There may be a bias due to the limited amount of cases enrolled. On the other hand, the difference has an implication for heterogeneity of RCC.

The top 2 most common altered genes in our study were *ABCB1* (50%) and *UGT1A1* (42.87%). *ABCB1*, ATP binding cassette subfamily B member 1, is a membrane-associated protein encoding gene. ABC proteins transport various molecules across extra- and intra-cellular membranes. This protein is a member of the MDR/TAP subfamily. Members of the MDR/TAP subfamily are involved in multidrug resistance. The protein encoded by this gene is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. Non-silent mutations of this gene were responsible for increased drug accumulation
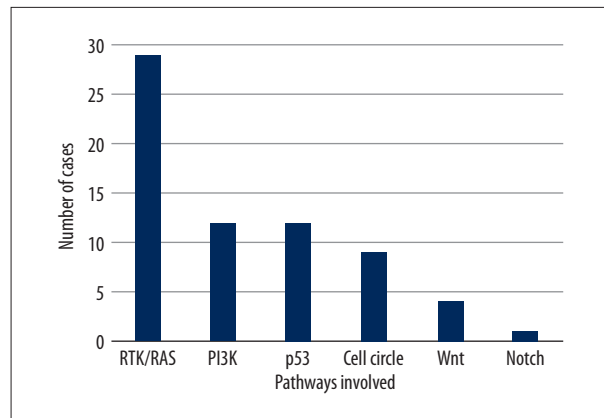
Figure 3. The number of clear cell renal cell carcinoma (ccRCC) cases involved in each oncogenic pathway in the 2 cohorts (n=84). Six oncogenic pathways were involved and the most common one was the RTK/RAS pathway.

and thus mediates the development of adverse events [16]. *UGT1A1*, UDP glucuronosyltransferase family 1 member A1, is a gene encodes a UDP-glucuronosyltransferase, an enzyme of the glucuronidation pathway that transforms small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites. Half of the ccRCC patients in our study possess *ABCB1* alterations, which may partially explain the chemoresistance property of this disease [17]. The polymorphism of *UGT1A1* was associated with the plasma level of targeted agents in RCC [18]. *ABCB1* also showed a marginal difference between the 2 groups with different TMB levels which may be of no significance.

In cohort 1, the patient ID 2 revealed the longest DFS of 14 months. This patient was among those 3 patients (ID 2, ID 14, and ID 15) who used axitinib for treatment after surgery, nevertheless ID 2 patient had a much longer DFS than the other 2 patients. Those 3 patients had similar performance status and TNM stage, except that patient ID 2 possessed a mutation in *VHL* which was predicted resulting in the dysfunction of this tumor suppressor gene. ccRCC with mutated *VHL* was highly vascularized [19], therefore, had a good response towards anti-angiogenetic agents. Non-silent mutation of *VHL* may be a predictor for the outcome of ccRCC treated with axitinib.

Six oncogenic pathways were involved in ccRCC cases in our study, which may reflect the reported complexity of ccRCC development and highly heterogeneity of ccRCC [20],

To our knowledge, this was the largest study of RCC genomic alterations and its relationship with TMB levels for Chinese patients. And differences in RCC genomics were found between Chinese patients and other races. There were limitations of this study. First, this was a retrospective study and the clinical information of patients especially those in cohort 2

was incomplete. Second, the majority of patients involved were ccRCC. Only 2 PRCC and one ChRCC were collected in cohort 1. Third, the therapeutic regimens and follow-ups were not well conducted, leaving analyses of genomic alterations with therapeutic effects or survival data unavailable.

Conclusions

The genomic alterations of Chinese RCC patients were different from that in TCGA and COSMIC. No significant genomic alterations were found correlating to TMB levels in ccRCC. Non-silent mutation of *VHL* may be a predictor for the outcome of ccRCC treated with axitinib.

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