LETTER TO THE EDITOR

Somatic mutations in renal cell carcinomas from Chinese patients revealed by targeted gene panel sequencing and their associations with prognosis and PD-L1 expression

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Dear Editor,

Renal cell carcinoma (RCC) is among the most common human cancers in the United States, with approximately 63,990 new patients and 14,400 deaths annually [1]. However, RCC is not among the top 10 malignancies in China in terms of incidence and mortality [2]. The clinical and molecular features of RCC differ among distinct pathological types, mainly clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (PRCC), and chromophobe renal cell carcinoma (ChRCC). The most common subtype of RCC is ccRCC worldwide. According to The Cancer Genome Atlas (TCGA), the somatic mutation landscape of RCC has been revealed by whole-exome sequencing (WES) or whole-genome sequencing (WGS). In our previous WES study, we validated most of the significantly mutated genes reported by the TCGA and identified several novel somatically altered genes [3]. The TCGA study showed that only somatic mutations in BRCA1-associated protein 1 (BAP1) were associated with patients’ poor survival outcomes among all significantly mutated genes [4]. In our previous WES study, BAP1 was somatically mutated in 2 of 15 ccRCC samples [3]. Nevertheless, all of these RCC patients lacked follow-up information. Hence, further analysis is needed to determine whether there are any somatically mutated genes associated with the prognosis of Chinese patients with RCC. However, WES or WGS is time-consuming and costly. Furthermore, compared with targeted sequencing, WES was more likely to generate false positives and false negatives due to insufficient base coverage [5].

In recent years, immunotherapy has played an increasingly important role in the treatment of advanced RCC and other malignancies. Based on the current understanding, programmed death-1 (PD-1) can combine with programmed death-ligand 1 (PD-L1) to confine T cell activity in the tumor microenvironment, and inhibition of the PD-1/PD-L1 pathway can increase the anti-tumor immune response [6]. Nivolumab, a PD-1 immune checkpoint inhibitor, has been validated for the treatment of advanced RCC based on the overall survival (OS) benefit [7]. A recent study has shown that PD-L1 expression was a predictive factor in terms of response and OS benefit from nivolumab plus ipilimumab combination therapy or nivolumab monotherapy as a second-line treatment for advanced RCC [8]. In our previous study, we identified several somatically mutated genes associated with PD-L1 expression in RCC tumor cells, including CSPG4, DNAH11, INADL, and TMPRSS13 [3]. However, the sample size in the previous study was only 26 specimens, which was a little bit small. In the present study, we aimed to validate these discoveries with a larger sample size and investigate the association between somatic mutations and PD-L1 expression in RCC tumor cells.

In the present study, formalin-fixed paraffin-embedded (FFPE) RCC specimens from 40 patients were investigated using immunohistochemistry (IHC) and targeted sequencing with a multi-gene panel.

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sequencing. We designed a gene panel comprising of 173 genes, which contained the newly identified somatically mutated genes, the genes somatically mutated in at least two samples in our previous WES study, and the recurrently mutated genes reported in the TCGA and Catalogue of Somatic Mutations in Cancer (COSMIC) database. The sequencing depth was set to 500×. All the identified somatic mutations were annotated using Annovar [9]. The functional significance of missense mutations was predicted via several algorithms, including SIFT, PolyPhen2 HDIV, PolyPhen2 HVAR, LRT, MutationTaster, MutationAssessor, and FATHMM. The somatic mutations scored with at least two algorithms as deleterious were deemed as deleterious variants. Other variants, including nonsense, frameshift, and canonical ±1 or ±2 splice site mutations, were considered to be pathogenic according to the guidelines of the American College of Medical Genetics (ACMG) [10]. Among these 40 RCC patients, 27 were males and 13 were females, with a median age of 57 years (range 22–76 years). The median follow-up for these 40 patients was 74 months (range 15–86 months). Details of their clinicopathological information are listed in Table 1.

Among all the significantly mutated genes in ccRCC from the TCGA database, VHL, PBRM1, SETD2, KDM5C, PTEN, BAP1, MTOR, and TP53 were the eight most significantly mutated genes [4]. All the eight genes were validated in the present study, whereas only six were validated in our previous WES study [3]. In the present study, VHL was somatically mutated in 10 ccRCC specimens, including five frameshift mutations, namely, p. K159fs, p. L135fs, p. P2fs, p. S183fs, and p. R58fs, all of which had not been reported previously and were deemed to be very strong evidence of pathogenicity. PBRM1 was somatically mutated in 7 ccRCC specimens, 5 PRCC specimens, and 3 ChRCC specimens. Most of the mutations in PBRM1 were frameshift mutations, which had not been reported previously and were predicted to be deleterious. The tumor mutation burden (TMB) of the 40 RCC specimens was calculated based on the custom-designed 173-gene panel. The TMB was significantly higher in RCC specimens with somatically mutated PBRM1 than in those without somatically mutated PBRM1 (P = 0.020). The sequencing depth in the present study was higher than that in our previous WES study. Consequently, more somatic mutations in each single specimen were revealed in the present study than in the TCGA data. There was usually more than one type of mutation identified in a single gene in multiple specimens. For instance, BAPI was somatically mutated in 3 ccRCC specimens in the present study, namely a frameshift deletion (p. S432fs) and insertion (p. P462fs) in sample 9, a deletion–insertion mutation [p. E642_ l643delins (39)] in sample 13, and a frameshift insertion (p. P339fs) and deletion–insertion mutation [p. I191_ D192delins (18)] in sample 20. Mutated BAPI or loss of BAPI expression was reported to be associated with poor outcome in ccRCC [4, 11]. However, no significant association between BAPI and prognosis was found in the present study.

In our previous WES study, we identified several newly somatically mutated genes, including HGC6.3, DDX51, NWD2, CDC42EP1, NPIP5, HSCB, HMCN2, and PCDHB9 in ccRCC; DEPDC4, PNLIP, SARDH, and ZAN in PRCC; and KRTAP4-8 in ChRCC [3]. All of these genes were enrolled in our custom-designed gene panel for further investigation with a larger sample size. As such, most of these newly identified somatically mutated genes were validated in the present study, except for HMNC2 and PCDHB9 in ccRCC and DEPDC4 and ZAN in PRCC. Three somatic mutations in DEPDC4 were identified in ccRCC specimens, namely 2 frameshift deletions (p. F150fs and p. R215fs) and 1 deletion–insertion [p. E147_L148delins (8)], all of which were predicted to be deleterious. Among the 40 RCC patients with complete follow-up information, univariate survival analysis with log-rank tests revealed that the disease-free survival (DFS) was shorter in patients with the maximum diameter of tumor > 7 cm than in patients with the maximum diameter of tumor ≤ 7 cm (P = 0.003) and shorter in patients with American Joint Committee on Cancer (AJCC) stage III than in patients with AJCC stage I–II (P < 0.001). In addition, we found a slight trend towards an association between DFS and somatically mutated DDX51 (P = 0.144). The three variables with P < 0.15 were all enrolled in the multivariate Cox regression survival analysis, which showed that somatically mutated DDX51 (P = 0.017) and AJCC stage III (P = 0.006) were independent risk factors for DFS among RCC patients (Table 2 and Fig. 1). However, no significant association between somatically mutated genes and OS was found in the present study. Among the 20 ccRCC specimens in the present study, DDX51 was somatically mutated in 5 specimens with six mutations, namely, a deletion–insertion mutation [p. K611_V612delins (35)] in 1 specimen, a missense mutation (p. S116N) in 2 specimens, two frameshift insertion mutations (p. G147fs and p. H28fs) in 1 specimen, and a frameshift deletion (p.A273fs) in 1 specimen. Notably, the frameshift deletion (p. A273fs) was located in the DEAD protein domain of DDX51.
Table 1 The clinicopathological information of 40 RCC patients

| Sample ID | Gender | Age  | Subtype   | Tumor grade | TNM stage | AJCC stage | OS (months) | DFS (months) | Outcome         |
|-----------|--------|------|-----------|-------------|-----------|------------|-------------|--------------|----------------|
| 1         | Male   | 76   | ccRCC     | G2          | T3aN0M0   | III        | 34          | 34           | Death          |
| 2         | Male   | 74   | ccRCC     | G2          | T1aN0M0   | I          | 32          | 32           | Death          |
| 3         | Male   | 31   | ccRCC     | G3          | T3aN0M0   | III        | 63          | 63           | Death          |
| 4         | Male   | 74   | ccRCC     | G2          | T1aN0M0   | I          | 62          | 62           | Death          |
| 5         | Male   | 54   | ccRCC     | G2          | T1aN0M0   | I          | 57          | 57           | Death          |
| 6         | Male   | 62   | ccRCC     | G2          | T1aN0M0   | I          | 76          | 64           | Survival (metastasis) |
| 7         | Female | 40   | ccRCC     | G3          | T3bN0M0   | III        | 74          | 24           | Survival (metastasis) |
| 8         | Female | 57   | ccRCC     | G2          | T1aN0M0   | I          | 74          | 24           | Survival (metastasis) |
| 9         | Male   | 56   | ccRCC     | G2          | T3aN0M0   | III        | 72          | 9            | Survival (metastasis) |
| 10        | Male   | 59   | ccRCC     | G3          | T1bN0M0   | I          | 71          | 12           | Survival (metastasis) |
| 11        | Male   | 55   | ccRCC     | G1          | T1bN0M0   | I          | 74          | 74           | Survival       |
| 12        | Male   | 62   | ccRCC     | G2          | T1bN0M0   | I          | 74          | 74           | Survival       |
| 13        | Male   | 54   | ccRCC     | G2          | T1aN0M0   | I          | 74          | 74           | Survival       |
| 14        | Male   | 60   | ccRCC     | G2          | T1bN0M0   | I          | 74          | 74           | Survival       |
| 15        | Male   | 48   | ccRCC     | G1          | T1aN0M0   | I          | 74          | 74           | Survival       |
| 16        | Male   | 68   | ccRCC     | G2          | T1bN0M0   | I          | 73          | 73           | Survival       |
| 17        | Male   | 48   | ccRCC     | G2          | T1aN0M0   | I          | 73          | 73           | Survival       |
| 18        | Male   | 73   | ccRCC     | G1          | T1aN0M0   | I          | 73          | 73           | Survival       |
| 19        | Female | 58   | ccRCC     | G2          | T1aN0M0   | I          | 72          | 72           | Survival       |
| 20        | Male   | 48   | ccRCC     | G2          | T1aN0M0   | I          | 72          | 72           | Survival       |
| 21        | Male   | 49   | PRCC      | G2          | T2N0M0    | II         | 15          | 15           | Death          |
| 22        | Female | 66   | PRCC      | G2          | T3aN1M0   | III        | 37          | 37           | Death          |
| 23        | Male   | 70   | PRCC      | G2          | T3aN0M0   | III        | 66          | 66           | Death          |
| 24        | Male   | 63   | PRCC      | G2          | T3bN0M0   | III        | 29          | 29           | Death          |
| 25        | Male   | 65   | PRCC      | G2          | T1aN0M0   | I          | 49          | 49           | Death          |
| 26        | Female | 22   | PRCC      | G1          | T1aN0M0   | I          | 76          | 76           | Survival       |
| 27        | Female | 60   | PRCC      | G2          | T1aN0M0   | I          | 74          | 74           | Survival       |
| 28        | Male   | 69   | PRCC      | G2          | T1aN0M0   | I          | 73          | 73           | Survival       |
| 29        | Male   | 59   | PRCC      | G2          | T1aN0M0   | I          | 71          | 71           | Survival       |
| 30        | Male   | 58   | PRCC      | G2          | T1aN0M0   | I          | 69          | 69           | Survival       |
| 31        | Female | 49   | ChRCC     | NA          | T2N0M0    | II         | 86          | 86           | Survival       |
| 32        | Male   | 64   | ChRCC     | NA          | T1aN0M0   | I          | 85          | 85           | Survival       |
| 33        | Female | 37   | ChRCC     | NA          | T1aN0M0   | I          | 85          | 85           | Survival       |
| 34        | Male   | 36   | ChRCC     | NA          | T1bN0M0   | I          | 84          | 84           | Survival       |
| 35        | Female | 54   | ChRCC     | NA          | T1aN0M0   | I          | 84          | 84           | Survival       |
| 36        | Female | 52   | ChRCC     | NA          | T1aN0M0   | I          | 82          | 82           | Survival       |
| 37        | Female | 75   | ChRCC     | NA          | T1aN0M0   | I          | 78          | 78           | Survival       |
| 38        | Male   | 40   | ChRCC     | NA          | T1bN0M0   | I          | 77          | 77           | Survival       |
| 39        | Female | 36   | ChRCC     | NA          | T1bN0M0   | I          | 77          | 77           | Survival       |
| 40        | Female | 49   | ChRCC     | NA          | T1bN0M0   | I          | 76          | 76           | Survival       |

RCC renal cell carcinoma, TNM tumor-node metastasis stage, AJCC American Joint Committee on Cancer, OS overall survival, DFS disease-free survival, ccRCC clear cell renal cell carcinoma, PRCC papillary renal cell carcinoma, ChRCC chromophobe renal cell carcinoma, NA not available

* The 7th edition of the AJCC Cancer Staging Manual was used
The missense mutation in \( DDX51 \) in both specimens was predicted to be benign or neutral, whereas the deletion–insertion mutation and three frameshift mutations were most likely to be deleterious according to the ACMG guidelines. Furthermore, somatic mutations in \( DDX51 \) were also identified in two other RCC subtypes, including a frameshift deletion (p. R519fs) in PRCC predicted to be deleterious and a missense mutation (p. P123R) in ChRCC predicted to be benign or neutral.

In our previous study, PD-L1 expression in tumor cells was detected in 6 (23%) of 26 RCC specimens: 3 ccRCC specimens, 2 PRCC specimens, and 1 ChRCC specimen [3]. In the present study, PD-L1 expression in tumor cells was detected in 6 (15%) of the 40 RCC samples: 1 ccRCC sample, 4 PRCC samples, and 1 ChRCC sample (Fig. 3). Combined with the 26 RCC specimens investigated in our previous study, PD-L1 expression in tumor cells was positive in 4 (11%) of 35 ccRCC specimens. Nevertheless, only mutated \( RAD21 \) and \( BAP1 \) were associated with PD-L1 expression in tumor cells. Among the 35 ccRCC specimens (15 from our previous WES study [3] and 20 in the present study), Fisher’s exact test revealed that the PD-L1-positive rate in tumor cells was higher in specimens with somatically mutated \( RAD21 \) (\( P = 0.002 \)) and \( BAP1 \) (\( P = 0.006 \)) than in specimens without those mutated genes. The somatic mutations in \( BAP1 \) (p. P352fs, p. H193Q, p. S432fs, and p. P462fs) and \( RAD21 \) (p. F2L, p. F304S, p. R402fs, and p. L515fs) detected in the 3 PD-L1-positive ccRCC samples were all predicted to be deleterious.

![Figure 1](image1.png)

**Fig. 1** The Kaplan–Meier disease-free survival (DFS) curves of 40 renal cell carcinoma (RCC) patients: a Survival curves of patients with or without mutated DEAD-box helicase 51 (DDX51); b survival curves of patients with American Joint Committee on Cancer (AJCC) stages I–II or III

![Figure 2](image2.png)

**Fig. 2** Mutation Mapper interprets mutations with protein domains of DEAD-box helicase 51 (DDX51). The mutations are presented by circles and colors: green (missense), black (frameshift), brown (stop-gain)
In conclusion, RCC patients with somatically mutated PBRM1 tend to have higher TMB than those without it. The somatically mutated DDX51 is an independent risk factor for DFS among RCC patients and could be a new candidate gene for predicting the prognosis of RCC. The somatically mutated RAD21 and BAP1 are associated with PD-L1 expression in ccRCC tumor cells and might serve as a potential predictor of the response to immunotherapy with PD-1/PD-L1 inhibitors in ccRCC patients.

Abbreviations
RCC: renal cell carcinoma; ccRCC: clear cell renal cell carcinoma; PRCC: papillary renal cell carcinoma; ChRCC: chromophobe renal cell carcinoma; TCGA: The Cancer Genome Atlas; WES: whole-exome sequencing; WGS: whole-genome sequencing; PD-1: programmed death-1; PD-L1: programmed death-ligand 1; OS: overall survival; FFPE: formalin-fixed paraffin-embedded; AJCC: American Joint Committee on Cancer; IHC: immunohistochemistry, COSMIC: Catalogue of Somatic Mutations in Cancer; ACMG: American College of Medical Genetics; TMB: tumor mutation burden; DFS: disease-free survival; BAP1: BRCA1-associated protein 1; DDX51: DEAD-box helicase S1.

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Authors’ contributions
YX and JL contributed to the acquisition of whole data independently and presented the same results. JW, HZ, and JL were responsible for DNA extraction, library preparation and immunohistochemical assays. JW and ZX were involved in the diagnosis and the recruitment of the patients in our affiliated hospitals and follow-up study. YX, HZ, and JW contributed to statistical analysis and data interpretation. ZX and RX contributed to revising it critically for important intellectual content. ZX, JX, and RX designed and organized the study. JW drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All the raw data generated and analyzed during this study are available from the corresponding author on reasonable request.
Ethics approval and consent to participate
The study protocol conformed to the 1975 Declaration of Helsinki and all experiments involving human tissues and clinical data were performed in accordance with relevant guidelines. The present study was approved by the biomedical research ethics committee of Peking University First Hospital. Written informed contents were acquired from all the patients.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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