Clinicopathological and prognostic impact of somatic mutations in Chinese patients with clear cell renal cell carcinoma

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Background: The study of the genomic landscape of Chinese clear cell renal cell carcinoma (ccRCC) entered its nascence in recent years, and the clinical relevance of individual genes in Chinese ccRCC has not yet been researched. The study aimed to explore the relationships between somatic mutations and clinical behaviors in Chinese ccRCC.

Methods: Tumor tissue samples were obtained from 105 Chinese patients with ccRCC and deep sequencing targeting 556 cancer genes was performed. Correlation analysis, receiver operator characteristic (ROC) analysis and survival analysis were carried out using SPSS software.

Results: A total of 41 genes were used to investigate the relationship between genes and clinical behaviors. We found that different clinical indices were mutually correlated, and there were 12 genes associated with clinical indices. The Kaplan-Meier curves showed that high Fuhrman grade and metastatic disease at diagnosis were significantly associated with poor prognosis. Mutations in *BAP1*, *PTEN*, *ERBB2*, *TP53*, *CDK8*, *TSC1*, *SETD2*, or *SPEN* were significantly associated with poor prognosis, consistent with the results of The Cancer Genome Atlas (TCGA) cohort. Mutation of *BTG1* occurred much more frequently in Chinese ccRCC (10.5%) than in the TCGA cohort (0.60%), and it was associated with a better prognosis.

Conclusions: A total of 8 genes (*BAP1*, *PTEN*, *ERBB2*, *TP53*, *CDK8*, *TSC1*, *SETD2*, and *SPEN*) were found to be associated with poor prognosis of ccRCC, and a new gene (*BTG1*) was possibly associated with the good prognosis of Chinese ccRCC.

Keywords: Clear cell renal cell carcinoma (ccRCC), targeted next-generation sequencing (targeted NGS), correlation analysis, survival analysis, prognostic biomarker

Introduction

Renal cell carcinoma (RCC) is one of the most common malignant neoplasm arising from the kidney, representing about 2% of all human malignancies, with 403,262 new cases and 175,098 related deaths estimated worldwide in 2018 (1). Clear-cell (cc) RCC is the most common subtype, accounting for up about 80% of all RCC cases (2). TNM stage and Fuhrman grade are the most commonly used predictors of clinical outcome for patients with ccRCC. However, patients with similar clinical features may have diverse outcomes. A commonly used stratification tool, Memorial Sloan Kettering Cancer Center (MSKCC) risk model, allows stratification of patients with RCC into one of three categories (favourable, intermediate, or poor prognosis) (3). One of the main limitations of this model is that most patients were typically classified as intermediate risk. Thus, there is a need to add prognostic value to the current stratification tools, which has been initially achieved with the use of validated genetic biomarkers (4). In addition, the promising curative effect of targeted therapies drove the studies on genomic research on ccRCC, intending to discover the putative targets in RCC pathogenesis (5,6). With the increasing treatment methods for RCC, selecting the right therapy for the right individual becomes crucial. Fortunately, the use of genomic research in clinical trials has begun to clarify the predictive or prognostic roles of certain genomic alterations in guiding RCC outcomes (7).

Genomic research has been conducted extensively in ccRCC, in which the mutation frequency of *VHL*, *PBRM1*, *SETD2*, *BAP1*, *KDM5C*, *TP53*, and *MTOR* is 52%, 30%, 13%, 13%, 7%, 7%, and 7%, respectively. Many studies have focused on identifying the clinical relevance of individual genes (7-10). Growing evidence supports the prognostic value of chromatin remodeling genes, such as *PBRM1*, *BAP1*, *SETD2*, and *EZH2*, as well as the DNA damage repair (DDR) genes (7). Most of the studies were performed on patients from Western countries, and little is known about the genomic landscape of Chinese ccRCC and how it differs from that of Western cohorts. In recent years, the genomic landscape of Chinese ccRCC has been depicted by limited studies (11-13); however, the clinical relevance of individual genes in Chinese ccRCC has not been studied yet.

In this study, we performed deep sequencing targeting 556 cancer genes on 105 Chinese patients with ccRCC and investigated the clinicopathological significance of frequently mutated genes.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tau-20-1410).

Methods

Participants and samples

Tumor tissue samples for genetic testing were taken from 105 participants with ccRCC who underwent surgery at the Peking University Third Hospital between August 2013 and March 2020, among which 103 patients were operated on after 2018. The treatments of these patients were randomized. We retrospectively collected the clinical and pathological data of these 105 participants. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by Peking University Third Hospital Medical Science Research Ethics Committee (No. M2018042). Written informed consent was provided by all participants.

We validated our findings using the dataset of ccRCC from The Cancer Genome Atlas (TCGA-KIRC) (August 8, 2019, update).

Targeted next-generation sequencing (NGS)

We extracted DNA from tissue samples, and the invasive tumor content was evaluated by pathologists, to ensure >50% of cells were tumor cells. The DNA was isolated using the DNeasy Tissue Kit (69504, QIAGEN, Venlo, Netherlands). Multi-gene panel targeted NGS including 556 genes was performed on Ion Torrent (Tongshu BioTech, Shanghai, China). The targeted libraries were constructed using NGS Fast DNA Library Prep Set (Thermo Fisher, Waltham, MA, USA). Sequencing data were analyzed by TorrentSuit-5.6 (3730XL, Life Technologies Corp., Carlsbad, CA, USA) with mutation calling using VarDict (14). The clinicopathological significance of 41 genes with mutation frequencies >3% was investigated.

Statistical analysis

Statistical analysis was performed using the software SPSS 25.0 (IBM Corp. Armonk, NY, USA). The correlations between different clinical indices, as well as the correlations between mutation status and clinical indices, were analyzed using crosstabs analysis. Fisher's exact test was

Table 1 The clinicopathological features of 105 Chinese patients with ccRCC

Items	Total (n=105)
Gender	
Male	82 (78.1%)
Female	23 (21.9%)
Age	
Median [range]	58 [26–83]
Clinical stage	
I	42 (40.0%)
II	5 (4.8%)
III	38 (36.2%)
IV	20 (19.0%)
T	
T1	43 (40.9%)
T2	5 (4.8%)
T3	55 (52.4%)
T4	2 (1.9%)
N	
N0	85 (81.0%)
N1	20 (19.0%)
M	
M0	85 (81.0%)
M1	20 (19.0%)
Fuhrman grade	
1	5 (4.8%)
2	62 (59.0%)
3	25 (23.8%)
4	13 (12.4%)
Infringement	
No	47 (44.8%)
Yes	58 (55.2%)
Necrosis	
No	79 (75.2%)
Yes	26 (24.8%)
Sarcomatoid	
No	95 (90.5%)
Yes	10 (9.5%)
Tumor thrombus	
No	80 (76.2%)
Yes	25 (23.8%)

ccRCC, clear cell renal cell carcinoma; T, tumor; N, node; M, metastasis.

used to determine the associations. A receiver operator characteristic (ROC) curve was used to evaluate the performance of mutation status to predict clinical outcomes. The Kaplan-Meier method was used to analyze disease-free survival (DFS) stratified by clinical indices or mutation status. Statistical significance was defined as two-tailed P values <0.05.

Results

Clinicopathologic characteristics of ccRCC

The clinicopathologic characteristics of ccRCC are summarized in *Table 1*. This cohort consisted of 82 (78.1%) males and 23 (21.9%) females. The participants' ages at diagnosis were 26–83 years, and the median age was 58 years. There were 42 (40.0%), 5 (4.8%), 38 (36.2%), and 20 (19.0%) participants in clinical stage I, II, III, and IV, respectively. Tumor sizes were 2–12.8 cm (median size, 6 cm), and there were 43 (40.9%), 5 (4.8%), 55 (52.4%), and 2 (1.9%) participants in stage T1, T2, T3, and T4, respectively. Metastatic disease was present in 34 (32.4%) participants at the time of diagnosis. There were 5 (4.8%), 62 (59.0%), 25 (23.8%), and 13 (12.4%) participants with Fuhrman grade 1, 2, 3, and 4, respectively. Among the 105 patients, 58 (55.2%) were infringement, 26 (24.8%) were necrosis, 10 (9.5%) were sarcomatoid, and 25 (23.8%) had tumor thrombi.

The interrelationship between different clinical indices of ccRCC

We studied the interrelationship between clinical indices, including gender, age at diagnosis, clinical stage, pathological T (pT) stage, metastasis at diagnosis, Fuhrman grade, infringement, necrosis, sarcomatoid, and tumor thrombus. As a result, we found that all clinical indices were correlated. As shown in *Figure 1*, the infringement is strongly associated with clinical stage and pT stage. The infringement rarely occured in lower stages (clinical stage I/II or pT1/2), but did so almost always in higher stages (clinical stage III/IV or pT3/4) (Table S1). The infringement was also associated with Fuhrman grade, tumor thrombus, necrosis, sarcomatoid, and metastasis at diagnosis. Infringement occurred in most of the participants with tumor thrombus (96.0%, Table S2), necrosis (80.8%, Table S3), sarcomatoid (100%, Table S4), and metastasis at diagnosis (88.2%) (Table S5).

Figure 1 Correlation analysis between clinical indices (P<0.01). Red, positively correlated; blue, negatively correlated. Number, the correlation coefficients; blank, uncorrelated. pT, pathological T.

The result showed that the higher the Fuhrman grade, the higher the likelihood of infringement, tumor thrombus, necrosis, sarcomatoid, metastasis at diagnosis, and high stages (Table S6). Among the patients with Fuhrman grade 4, the incidence of infringement, tumor thrombus, necrosis, sarcomatoid, metastasis, high clinical stage, and high pT stage was 92.3%, 53.8%, 76.9%, 53.8%, 69.2%, 92.3%, and 92.3%, respectively (Table S6).

Metastasis at diagnosis was associated with clinical stage, pT, Fuhrman grade, infringement, necrosis, sarcomatoid, and tumor thrombi (*Figure 1*). Patients with metastasis at diagnosis were often already in high stages (94.1% in clinical stage III/IV and 91.2% in pT3/4, Table S3), and participants with high clinical stage or pT were predominantly metastatic at diagnosis (95.2% and 93.0%, respectively). Metastatic disease was found in most participants with tumor thrombus (80.0%, Table S2), necrosis (61.5%, Table S3), and sarcomatoid (80.0%, Table S4).

The participants with tumor thrombus usually had necrosis (44.0%) and high stages (96.0%), but there was no relationship between tumor thrombus and sarcomatoid carcinoma (Table S2). Patients with sarcomatoid lesion or necrosis had a high probability of infringement, metastasis, and high stage (Table S3, Table S4). In addition, we found that male ccRCC patients were distributed through different age groups, while female ccRCC patients were mainly >58 years (Table S7).
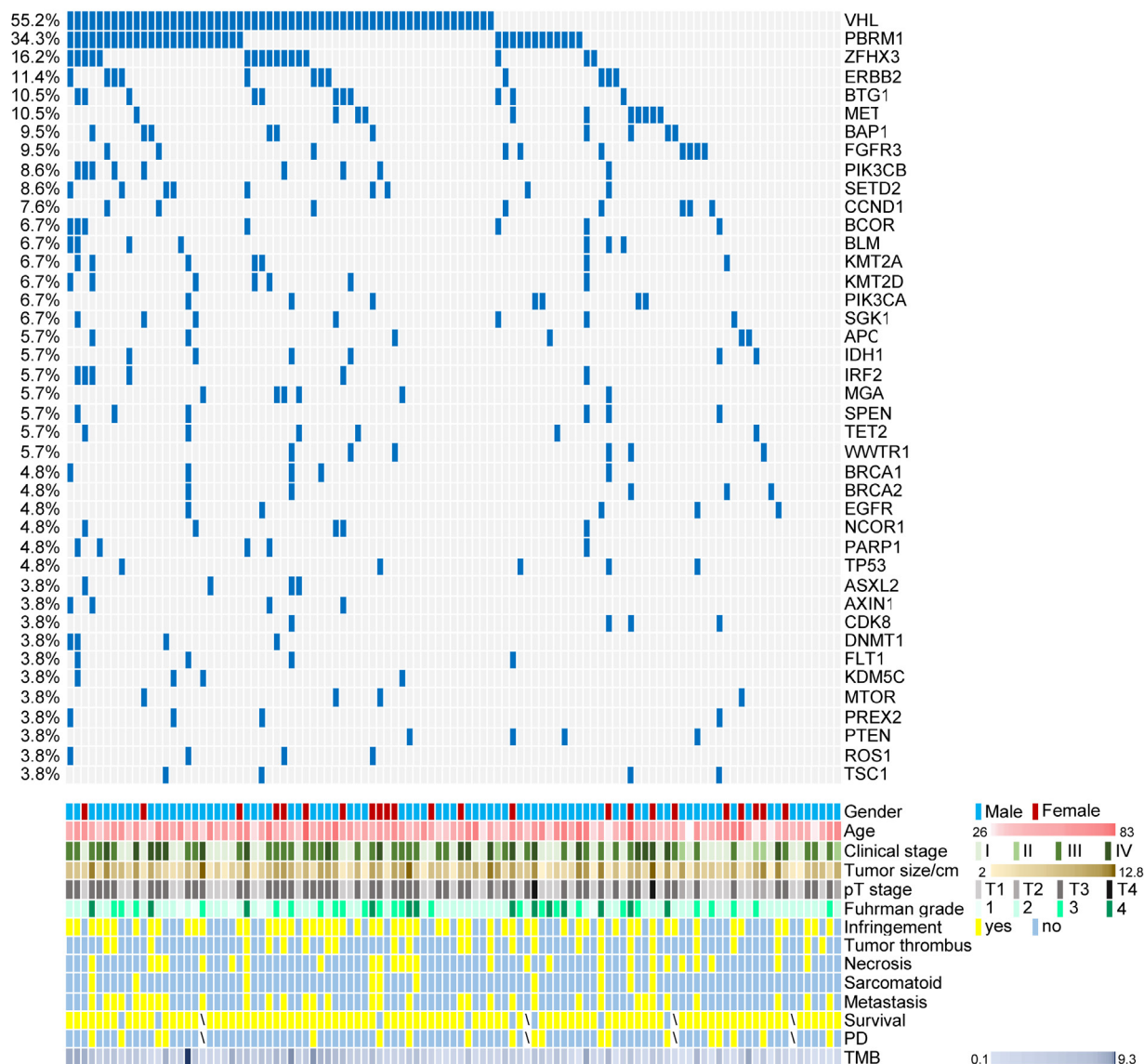
The relationship between genetic alterations and clinical indices of ccRCC

Among the 556 cancer genes, 212 genes were detected in this cohort. The most frequently altered genes were *VHL* (55.2%), *PBRM1* (34.3%), *ZFHX3* (16.2%), *ERBB2* (11.4%), *BTG1* (10.5%), *MET* (10.5%), *BAP1* (9.5%), *FGFR3* (9.5%), *PIK3CB* (8.6%), *SETD2* (8.6%), and *CCND1* (7.6%). A total of 41 genes with alteration frequencies >3% were used to investigate the relationship between genes and clinical behaviors (*Figure 2*). A total of 7 (*TP53, BAP1, PIK3CB, CCND1, WWTR1, MTOR,* and *PTEN*) were found to be significantly associated with at least one clinical index (P<0.05). Considering the relatively small size of our cohort, we expanded the P-value to 0.1, and an additional 5 genes (*BTG1, BLM, ERBB2, PARP1* and *MET*) were included (*Figure 3*).

The results showed that mutations in *BAP1, PIK3CB, WWTR1,* and *MTOR* occurred more frequently in female (13.0–26.1%) than male (1.2–6.1%) ccRCC (Table S8). Mutations in *TP53, BAP,1* and *PTEN* were usually found in instances of higher Fuhrman grade (Table S9). Mutations in *BAP1, ERBB2,* and *PARP1* were associated with the infringement (Table S10). Mutations of *ERBB2, BAP1,* and *PTEN* were seen more frequently in RCC patients with metastasis at diagnosis, while *BTG1* and *BLM* were mutated more frequently in RCC patients without metastasis at diagnosis (Table S11). Mutations of *BAP1* and *PARP1* were shown more frequently in higher pT stage (Table S12). More frequent mutation of *BAP1* and *MET* was revealed in RCC patients with sarcomatoid carcinoma (Table S13). Moreover, *BAP1* was mutated more frequently in higher clinical stage, *CCND1* was mutated more frequently in younger patients, *PTEN* was mutated more frequently in patients with tumor thrombus, and *BTG1* was rarely mutated in patients with necrosis (Tables S14–S17).

The combinations of genes associated with a single

Figure 2 The 41 genes with alteration frequencies >3% were used to investigate the relationship between genes and clinical behaviors. Each row represents a gene, each column represents a sample, and blue represents mutation. Gender, age, clinical stage, tumor size, pT stage, Fuhrman grade, infringement, tumor thrombus, necrosis, sarcomatoid, metastatic disease at diagnosis, survival, PD, and TMB are shown as patient annotations. PD, progressive disease; TMB, tumor mutation burden.

clinical index were used to explore their ability to predict clinical outcomes; as a result, the combinations improved the ability to predict clinical outcomes (*Tables 2-6*). Among participants with mutated *BAP1/PTEN/ERBB2*, 61.5% were metastatic at diagnosis [*Table 2*; area under the curve (AUC) =0.694, P=0.003, *Figure 4A*]; and patients with mutated *BLM/BTG1* had very low risk of metastasis (6.7%) (*Table 2*; AUC =0.65, P=0.064, *Figure 4B*). Those with mutated

BAP1/PTEN/TP53 mostly had higher Fuhrman grade (72.2%, *Table 3*; AUC =0.717, P=0.004, *Figure 4C*). Of the participants with mutated *BAP1/ERBB2/PARP1*, 87.5% were infringement (*Table 4*; AUC =0.709, P=0.002, *Figure 4D*), and 92.3% of patients with mutated *BAP1/ PARP1* had higher tumor node metastasis (TNM) stage (*Table 5*; AUC =0.717, P=0.012, *Figure 4E*). However, only 26.3% of patients with mutated *BAP1/MET* were

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Figure 3 Correlation analysis between mutations and clinical indices (P<0.1). Red, positively correlated; blue, negatively correlated. Number, the correlation coefficients; blank, uncorrelated. pT, pathological T.

Table 2 The relationship between combined genes and metastasis at diagnosis

	Metastasis at diagnosis			Phi coefficient	Fisher's P value
	Total (n=105)	No (n=71)	Yes (n=34)		
BAP1/PTEN/ERBB2 Mut				0.357	0.001
No	79	61/79 (77.2%)	18/79 (22.8%)		
Yes	26	10/26 (38.5%)	16/26 (61.5%)		
BLM/BTG1 Mut				-0.224	0.033
No	90	57/90 (63.3%)	33/90 (36.7%)		
Yes	15	14/15 (93.3%)	1/15 (6.7%)		

Mut, mutation.

Table 3 The relationship between combined genes and Fuhrman grade

	Fuhrman grade					Phi coefficient	Fisher's P value
	Total (n=105)	1 (n=5)	2 (n=62)	3 (n=25)	4 (n=13)		
BAP1/PTEN/TP53 Mut						0.466	<0.001
No	87	5/87 (5.7%)	57/87 (65.5%)	20/87 (23.0%)	5/87 (5.7%)		
Yes	18	0/18 (0%)	5/18 (27.8%)	5/18 (27.8%)	8/18 (44.4%)		

Mut, mutation.

Table 4 The relationship between combined genes and infringement

	Infringement			Phi coefficient	Fisher's P value
	Total (n=105)	No (n=47)	Yes (n=58)		
BAP1/ERBB2/PARP1 Mut				0.353	<0.001
No	81	44/81 (54.3%)	37/81 (45.7%)		
Yes	24	3/24 (12.5%)	21/24 (87.5%)		

Mut, mutation.

Table 5 The relationship between combined genes and pT stage

	pT stage			Phi coefficient	Fisher's P value
	Total (n=105)	T1/T2 (n=48)	T3/T4 (n=57)		
BAP1/PARP1 Mut				0.287	0.003
No	92	47/92 (51.1%)	45/92 (48.9%)		
Yes	13	1/13 (7.7%)	12/13 (92.3%)		

pT, pathological T; Mut, mutation.

Table 6 The relationship between combined genes and sarcomatoid

	Sarcomatoid			Phi coefficient	Fisher's P value
	Total (n=105)	No (n=95)	Yes (n=10)		
BAP1/MET Mut				0.269	0.016
No	96	81/96 (84.4%)	5/96 (5.2%)		
Yes	19	14/19 (73.7%)	5/19 (26.3%)		

Mut, mutation.

sarcomatoid type (*Table 6*; AUC =0.603, P=0.163, *Figure 4F*). Of the participants with mutated *PTEN*, 75% had tumor thrombus (AUC =0.766, P=0.072, *Figure 4G*). None of the patients with mutated *BTG1* were of the necrosis type (AUC =0.638, P=0.135, *Figure 4H*).

The relationship between genetic alterations/clinical indices and survival of ccRCC patients

Survival analysis was conducted to explore the relationships between DFS and clinical indices, individual genes with alteration frequencies >3%, and the potential predictors of clinical outcomes.

As shown in *Figure 5A,B*, higher Fuhrman grade and metastasis at diagnosis were associated with poorer prognosis (P<0.05). Necrosis, high clinical stage, high

TNM stage, and sarcomatoid appeared to be associated with poor prognosis, but not at a statistically significant level (Figure S1A,B,C,D). Age, gender, infringement, and tumor thrombus were not associated with prognosis (Figure S1E,F,G,H).

The Kaplan Meier curves of patients stratified by genes with or without mutation showed that participants with mutations in *TP53*, *CDK8*, and *TSC1* had a poorer prognosis (P<0.05, *Figure 5C,D,E*). Mutations in *SETD2*, *SPEN*, *BAP1*, *ERBB2*, and *PIK3CB* also showed associations with poor prognosis, but they were not statistically significant (Figure S2). Several genes seemed to be associated with a better prognosis, including *VHL*, *BTG1*, and *MET*; however, they were not statistically significant (Figure S2).

Moreover, relationships between the combination
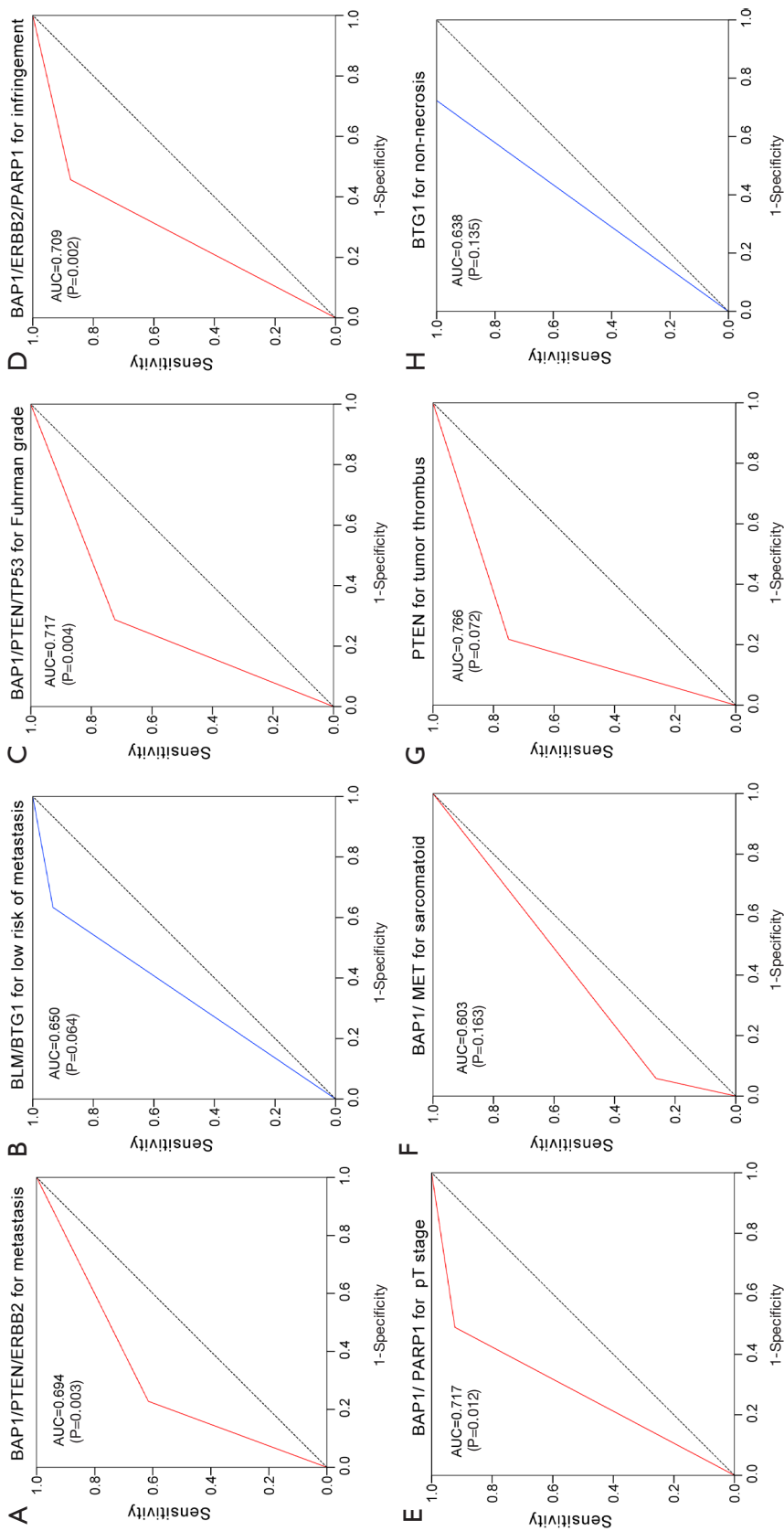
Figure 4 The performances of clinical behaviors were evaluated by ROC curves of different gene combination. Red, positively correlated; blue, negatively correlated. ROC, receiver operating characteristic; AUC, area under the ROC curve.
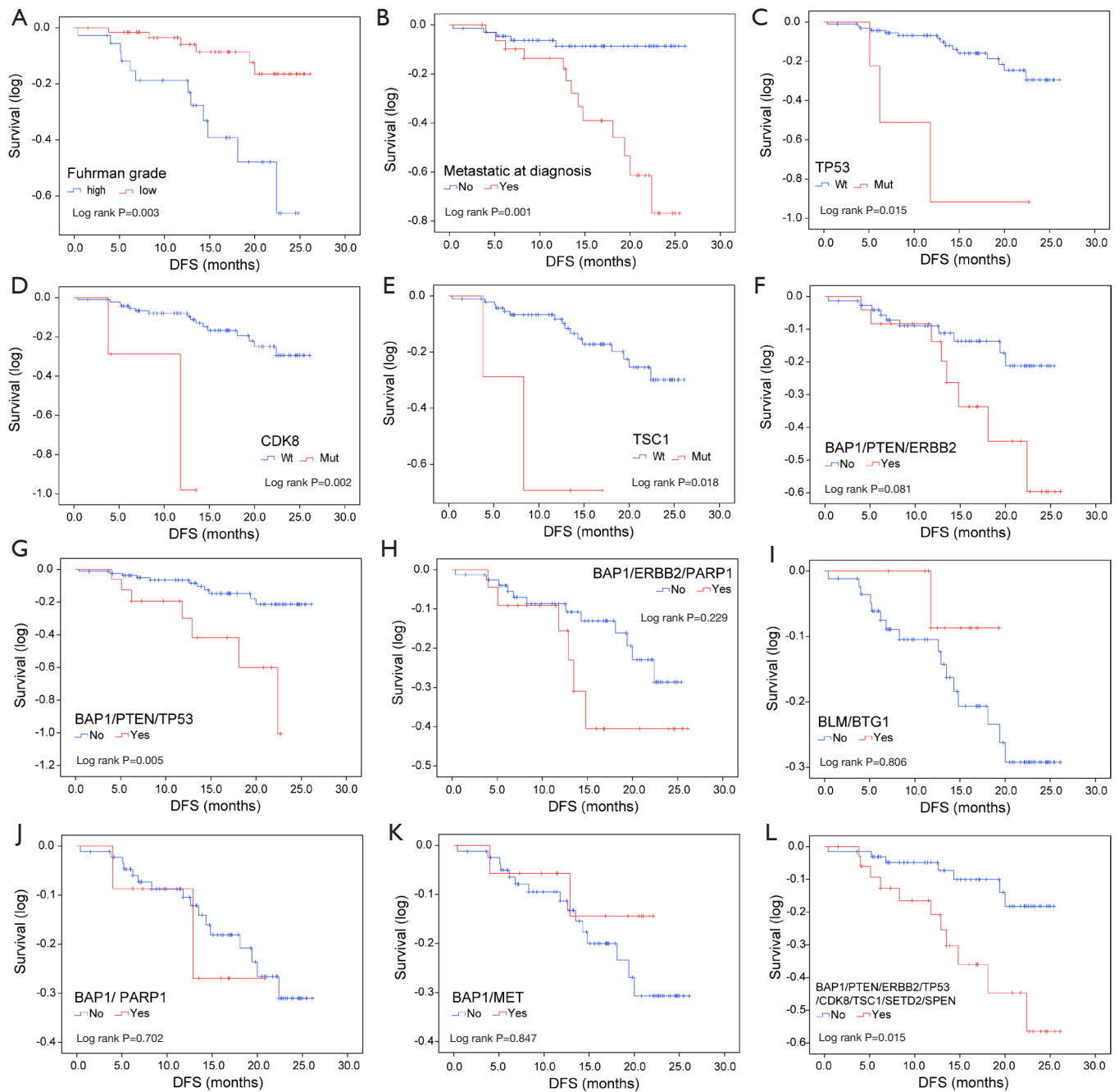
Figure 5 The relationship between genetic alterations/clinical indices and survival of ccRCC patients. (A,B) Kaplan-Meier curves for DFS of Chinese ccRCC patients with different clinical behaviors, (C,D,E) individual mutated genes, (F,G,H,I,J,K) combined genes associated with clinical behaviors, (L) combination of genes associated with poor prognosis. DFS, disease-free survival; ccRCC, clear cell renal cell carcinoma.

of genes and DFS were analyzed. As shown in *Figure 5F,G,H,I,J,K*, the predictors of metastasis at prognosis (mutant *BAP1/PTEN/ERBB2*) and the predictors of higher Fuhrman grade (mutant *BAP1/PTEN/TP53*) were associated with poor prognosis (P<0.1). The predictors of infringement (*BAP1/ERBB2/PARP1*) appeared to be associated with poor prognosis, and the predictors of low risk of metastasis at prognosis (*BLM/BTG1*) appeared to be associated with better prognosis, but this association was not statistically significant. However, the predictors of higher TNM stage (*BAP1/PARP1*) and sarcomatoid (*BAP1/MET*) were not associated with DFS.

Furthermore, we combined the predictors of metastasis at prognosis (*BAP1/PTEN/ERBB2*) and higher Fuhrman grade (*BAP1/PTEN/TP53*) with genes associated with prognosis, including *TP53*, *CDK8*, *TSC1*, *SETD2*, and *SPEN*, and analyzed their accumulated association with DFS. As shown in *Figure 5L*, patients with mutations of ≥1 of these genes (*BAP1*, *PTEN*, *ERBB2*, *TP53*, *CDK8*, *TSC1*, *SETD2*, and *SPEN*) had poor prognosis (P=0.015). The mutations of genes associated with prognosis of Chinese ccRCC were listed in Table S18.

We applied the TCGA-KIRC cohort to validate our findings. For the 8 genes associated with poor prognosis, similar mutation frequencies were seen in our cohort (*Figure 2*) and the TCGA-KIRC cohort (Figure S3), and their combination was significantly associated with poor prognosis (P=0.017), suggesting that these genes could predict the poor prognosis. The mutation frequencies of *BTG1* and *BLM* were much higher in our cohort (10.5% and 6.7%, respectively) (*Figure 2*) than they were in the TCGA-KIRC cohort (0.6% and 1.5%, respectively) (Figure S4A), and the results of survival analysis were comparable (*Figure 5I*, Figure S2, Figure S4B,C,D), showing that patients with mutant *BTG1* had a better prognosis.

Discussion

In this study, we enrolled 105 patients with ccRCC and investigated the interrelationships between different clinical indices, the relationships between genetic alterations and clinical indices, and the relationships between clinical indices/genetic alterations with DFS. The results showed that the clinical indices were interrelated (*Figure 1*), but only Fuhrman grade and metastasis were significantly associated with DFS (*Figure 5A,B*). A total of 12 genes were correlated with clinical indices (*Figure 3*), and the combinations of

genes were shown to predict clinical outcomes (*Figure 4*). Several genes were significantly associated with DFS (*Figure 5C,D,E,G*). Finally, a series of genes were proposed to have the potential to predict poor prognosis of ccRCC patients.

Most patients with tumor thrombus (96.0%), necrosis (80.8%), sarcomatoid (100%), metastasis at diagnosis (88.2%), or high Fuhrman grade (73.7%) had infringement, while only 41.1%, 36.2%, 17.2%, 51.7%, and 48.3% of patients with infringement had tumor thrombus, necrosis, sarcomatoid, metastasis at diagnosis, and high Fuhrman grade, respectively, indicating that infringement is not able to predict other clinical outcomes except for high stages (Tables S1-S7). High Fuhrman grade (grade 4) was usually accompanied by infringement (92.3%), metastasis at diagnosis (69.2%), necrosis (76.9%), and high stages (92.3%), while it was not associated with tumor thrombus or sarcomatoid. The interactions of different clinical indices are shown in Figure S5. Together with the results of survival analysis (*Figure 5A,B*, Figure S1), Fuhrman grade and metastasis had the most clinical relevance, while infringement had little clinical relevance. Besides Fuhrman grade and metastasis, necrosis, high stages, or sarcomatoid may be also associated with poor prognosis, although they were not significantly associated, possibly due to our relatively small sample size.

The clinicopathological significance of several genes was found. As a well-known gene in ccRCC (9,15-19), *BAP1* was found to be frequently mutated in females, patients with high Fuhrman grade, infringement, high stages, metastatic disease at diagnosis, and sarcomatoid lesions, but it was not significantly associated with poor prognosis (Figure S2). However, when *BAP1* was combined with other genes, it showed a significant association with poor prognosis (*Figure 5G,L*), indicating that *BAP1* was not the sole factor of poor prognosis. Another well studied gene in ccRCC, *PBRM1*, was reported to be associated with a better prognosis (9,20-25), while in other reports, it was not associated with survival (8,26-28). Our results showed that *PBRM1* was not associated with any clinical indices or DFS (Figure S2). It has been reported that *SETD2* is associated with a higher risk of disease recurrence and a shorter survival in ccRCC (16,29). In our study, the associations between *SETD2* and clinical indices were not found, but mutant *SETD2* was associated with shorter DFS (Figure S2). A previous study of chromophobe RCC showed that alterations in *TP53* and *PTEN* were associated with poor overall survival (OS), and these alterations were more

frequently observed in metastatic tissue samples compared with matched pT samples, suggesting a notable role in metastatic progression (30). In our study, *TP53* and *PTEN* were found to be significantly related to high Fuhrman grade, and *PTEN* was also significantly related to tumor thrombus and metastasis (*Figure 3*). Moreover, *TP53* and *PTEN* were associated with poor prognosis of patients with ccRCC (*Figure 5C,G,L*). Furthermore, we integrated the genes that were associated with prognosis, and studied the relationship between mutation status and DFS. The result showed a significant association between genes and DFS (*Figure 5L*), consistent with results of the TCGA cohort (Figure S6).

In addition, we found *BTG1* was negatively related to necrosis and metastatic disease at diagnosis (*Figure 3*), and it seemed to be associated with a better prognosis (Figure S2), which was consistent with results of the TCGA cohort (Figure S4B). However, *BTG1* was not significantly associated with prognosis in either of the 2 cohorts. As shown, the mutation frequency of *BTG1* was much higher in Chinese ccRCC participants (10.5%) than that in the TCGA cohort (0.60%). We suppose that mutation of *BTG1* may be specific to Chinese ccRCC and may be a good prognostic factor of ccRCC. A larger Chinese ccRCC cohort is needed to conduct a comprehensive and in-depth study of *BTG1*.

The function of the eight genes, which were found to be associated with poor prognosis of Chinese ccRCC in this study, has been elucidated in the literatures. *TP53* and *PTEN* are well known tumor suppressor genes that slow down cell division, repair DNA mistakes, or participate in the process of apoptosis or programmed cell death (7). *BAP1*, a H2A deubiquitinase, and *SETD2*, a histone H3 lysine 36 trimethylating enzyme are involved in chromosome remodelling. *BAP1* participates in homologous recombination (HR) repair and prevents chromosomal instability through modulation of various substrates, including MCRS1 and INO80. *SETD2* contributes to the maintenance of genome integrity through suppression of replication stress and promotion of HR repair, and contributes to DDR. *ERBB2*, a member of the epidermal growth factor receptor (EGFR) family with intrinsic protein tyrosine kinase activity, is involved in signal transduction, cell proliferation, development, differentiation, migration and tumor formation (31). CDK8 is a nuclear serine-threonine kinase that functions as a transcriptional regulator. *TSC1* is a component of TSC complex that is implicated as a tumor suppressor and negatively regulates

mTORC1 signaling (32). *SPEN* encodes a hormone inducible transcriptional repressor, with essential regulatory roles in transcriptional repression (33). *BTG1*, which might be associated with good prognosis of Chinese ccRCC, may inhibit cell growth and promote apoptosis by interacting with PRMT1 in RCC (34).

Conclusions

A total of 8 genes (*BAP1*, *PTEN*, *ERBB2*, *TP53*, *CDK8*, *TSC1*, *SETD2*, and *SPEN*) were found to be associated with poor prognosis of Chinese ccRCC, and a new gene (*BTG1*) was possibly associated with good prognosis of Chinese ccRCC.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by Peking University Third Hospital Medical Science Research Ethics Committee (No. M2018042). Written informed consent was provided by all participants.

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Table S1 The interrelationship between infringement and other clinical indices of ccRCC

	Infringement		Phi coefficient	Fisher's P-value
	No (n=47)	Yes (n=58)		
Clinical stage			0.923	<0.001
I/II	45/47 (95.7%)	2/58 (3.4%)		
III/IV	2/47 (4.3%)	56/58 (96.6%)		
pT			0.943	<0.001
T1/T2	46/47 (97.9%)	2/58 (3.4%)		
T3/T4	1/47 (2.1%)	56/58 (96.6%)		

ccRCC, clear cell renal cell carcinoma; pT, pathological T stage.

Table S2 The interrelationship between Fuhrman grade and other clinical indices of ccRCC

	Fuhrman grade				Phi coefficient	Fisher's P-value
	1 (n=5)	2 (n=62)	3 (n=25)	4 (n=13)		
Infringement					0.342	0.004
No	4/5 (80.0%)	33/62 (53.2%)	9/25 (36.0%)	1/13 (7.7%)		
Yes	1/5 (20.0%)	29/62 (46.8%)	16/25 (64.0%)	12/13 (92.3)		
Metastasis					0.336	0.007
No	5/5 (100%)	46/62 (74.2%)	16/25 (64.0%)	4/13 (30.8%)		
Yes	0/5 (0%)	16/62 (25.8%)	9/25 (36.0%)	9/13 (69.2%)		
Tumor thrombus					0.288	0.035
No	5/5 (100%)	50/62 (80.6%)	19/25 (76.0%)	6/13 (46.2%)		
Yes	0/5 (0%)	12/62 (19.4%)	6/25 (24.0%)	7/13 (53.8%)		
Necrosis					0.477	<0.001
No	4/5 (80.0%)	54/62 (87.1%)	18/25 (72.0%)	3/13 (23.1%)		
Yes	1/5 (20.0%)	8/62 (12.9%)	7/25 (28.0%)	10/13 (76.9%)		
Sarcomatoid					0.575	<0.001
No	5/5 (100%)	61/62 (98.4%)	23/25 (92.0%)	6/13 (46.2%)		
Yes	0/5 (0%)	1/62 (1.6%)	2/25 (8.0%)	7/13 (53.8%)		
Clinical stage					0.326	0.006
I/II	4/5 (80.0%)	32/62 (51.6%)	10/25 (40.0%)	1/13 (7.7%)		
III/IV	1/5 (20.0%)	30/62 (48.4%)	15/25 (60.0%)	12/13 (92.3%)		
pT					0.334	0.005
T1/T2	4/5 (80.0%)	33/62 (53.2%)	10/25 (40.0%)	1/13 (7.7%)		
T3/T4	1/5 (20.0%)	29/62 (46.8%)	15/25 (60.0%)	12/13 (92.3%)		

ccRCC, clear cell renal cell carcinoma; pT, pathological T stage.

Table S3 The interrelationship between metastatic disease at diagnosis and other clinical indices of ccRCC

| | Metastatic disease at diagnosis | | Phi coefficient | Fisher's P-value |
	No (n=71)	Yes (n=34)		
Infringement			0.459	<0.001
No	43/71 (60.6%)	4/34 (11.8%)		
Yes	28/71 (39.4%)	30/34 (88.2%)		
Clinical stage			0.541	<0.001
I/II	45/71 (63.4%)	2/34 (5.9%)		
III/IV	26/71 (36.6%)	32/34 (94.1%)		
pT			0.512	<0.001
T1/T2	45/71 (63.4%)	3/34 (8.8%)		
T3/T4	26/71 (36.6%)	31/34 (91.2%)		

ccRCC, clear cell renal cell carcinoma; pT, pathological T stage.

Table S4 The interrelationship between tumor thrombus and other clinical indices of ccRCC

| | Tumor thrombus | | Phi coefficient | Fisher's P-value |
	No (n=80)	Yes (n=25)		
Infringement			0.458	<0.001
No	46/80 (57.5%)	1/25 (4.0%)		
Yes	34/80 (42.5%)	24/25 (96.0%)		
Metastasis			0.569	<0.001
No	66/80 (82.5%)	5/25 (20.0%)		
Yes	14/80 (17.5%)	20/25 (80.0%)		
Necrosis			0.249	0.016
No	65/80 (81.3%)	14/25 (56.0%)		
Yes	15/80 (18.8%)	11/25 (44.0%)		
Clinical stage			0.458	<0.001
I/II	46/80 (57.5%)	1/25 (4.0%)		
III/IV	34/80 (42.5%)	24/25 (96.0%)		
pT			0.468	<0.001
T1/T2	47/80 (58.8%)	1/25 (4.0%)		
T3/T4	33/80 (41.3%)	24/25 (96.0%)		

ccRCC, clear cell renal cell carcinoma; pT, pathological T stage.

Table S5 The interrelationship between necrosis and other clinical indices of ccRCC

	Necrosis		Phi coefficient	Fisher's P-value
	No (n=79)	Yes (n=26)		
Infringement			0.295	0.003
No	42/79 (53.2%)	5/26 (19.2%)		
Yes	37/79 (46.8%)	21/26 (80.8%)		
Metastasis			0.357	<0.001
No	61/79 (77.2%)	10/26 (38.5%)		
Yes	18/79 (22.8%)	16/26 (61.5%)		
Clinical stage			0.339	0.001
I/II	43/79 (54.4%)	4/26 (15.4%)		
III/IV	36/79 (45.6%)	22/26 (84.6%)		
pT			0.349	<0.001
T1/T2	44/79 (55.7%)	4/26 (15.4%)		
T3/T4	35/79 (44.3%)	22/26 (84.6%)		

ccRCC, clear cell renal cell carcinoma; pT, pathological T stage.

Table S6 The interrelationship between sarcomatoid and other clinical indices of ccRCC

	Sarcomatoid		Phi coefficient	Fisher's P-value
	No (n=95)	Yes (n=10)		
Infringement			0.292	0.002
No	47/95 (49.5%)	0/10 (0%)		
Yes	48/95 (50.5%)	10/10 (100.0%)		
Metastasis			0.330	0.002
No	69/95 (72.6%)	2/10 (20.0%)		
Yes	26/95 (27.4%)	8/10 (80.0%)		
Necrosis			0.415	<0.001
No	77/95 (81.1%)	2/10 (20.0%)		
Yes	18/95 (18.9%)	8/10 (80.0%)		
Clinical stage			0.292	0.002
I/II	47/95 (49.5%)	0/10 (0%)		
III/IV	48/95 (50.5%)	10/10 (100.0%)		
pT			0.298	0.002
T1/T2	48/95 (50.5%)	0/10 (0%)		
T3/T4	47/95 (49.5%)	10/10 (100.0%)		

ccRCC, clear cell renal cell carcinoma; pT, pathological T stage.

Table S7 The interrelationship between gender and age of ccRCC

| | Gender | | Phi coefficient | Fisher's P-value |
	Male (n=82)	Female (n=23)		
Age			0.245	0.017
<58	42/82 (51.2%)	5/23 (21.7%)		
≥58	40/82 (48.8%)	18/23 (78.3%)		

ccRCC, clear cell renal cell carcinoma.

Table S8 The relationship between individual genes and gender

| | Gender | | Phi coefficient | Fisher's P-value |
	Male (n=82)	Female (n=23)		
BAP1			0.220	0.039
WT	77/82 (93.9%)	18/23 (78.3%)		
Mut	5/82 (6.1%)	5/23 (21.7%)		
PIK3CB			0.331	0.003
WT	79/82 (96.3%)	17/23 (73.9%)		
Mut	3/82 (3.7%)	6/23 (26.1%)		
WWTR1			0.266	0.02
WT	80/82 (97.6%)	19/23 (82.6%)		
Mut	2/82 (2.4%)	4/23 (17.4%)		
MTOR			0.255	0.032
WT	81/82 (98.8%)	20/23 (87.0%)		
Mut	1/82 (1.2%)	3/23 (13.0%)		

WT, wild type; Mut, mutant.

Table S9 The relationship between individual genes and Fuhrman grade

	Fuhrman grade				Phi coefficient	Fisher's P-value
	1 (n=5)	2 (n=62)	3 (n=25)	4 (n=13)		
TP53					0.296	0.067
WT	5/5 (100%)	61/62 (98.4%)	21/25 (84.0%)	13/13 (100%)		
Mut	0/5 (0%)	1/62 (1.6%)	4/25 (16.0%)	0/13 (0%)		
BAP1					0.374	0.01
WT	5/5 (100%)	58/62 (93.5%)	24/25 (96.0%)	8/13 (61.5%)		
Mut	0/5 (0%)	4/62 (6.5%)	1/25 (4.0%)	5/13 (38.5%)		
PTEN					0.388	0.004
WT	5/5 (100%)	62/62 (100%)	24/25 (96.0%)	10/13 (76.9%)		
Mut	0/5 (0%)	0/62 (0%)	1/25 (4.0%)	3/13 (23.1%)		

WT, wild type; Mut, mutant.

Table S10 The relationship between individual genes and infringement

	Infringement		Phi coefficient	Fisher's P-value
	No (n=47)	Yes (n=58)		
BAP1			0.227	0.022
WT	46/47 (97.9%)	49/58 (84.5%)		
Mut	1/47 (2.1%)	9/58 (15.5%)		
ERBB2			0.203	0.061
WT	45/47 (95.7%)	48/58 (82.8%)		
Mut	2/47 (4.3%)	10/58 (17.2%)		
PARP1			0.201	0.063
WT	47/47 (100%)	53/58 (91.4%)		
Mut	0/47 (0%)	5/58 (8.6%)		

WT, wild type; Mut, mutant.

Table S11 The relationship between individual genes and metastasis at diagnosis

	Metastasis at diagnosis		Phi coefficient	Fisher's P-value
	No (n=71)	Yes (n=34)		
PTEN			0.181	0.099
WT	70/71 (98.6%)	31/34 (91.2%)		
Mut	1/71 (1.4%)	3/34 (8.8%)		
BTG1			-0.170	0.099
WT	61/71 (85.9%)	33/34 (97.1%)		
Mut	10/71 (14.1%)	1/34 (2.9%)		
BLM			-0.185	0.093
WT	64/71 (90.1%)	34/34 (100%)		
Mut	7/71 (9.9%)	0/34 (0%)		
BAP1				0.074
WT	67/71 (94.4%)	28/34 (82.4%)		
Mut	4/71 (5.6%)	6/34 (17.6%)		
ERBB2			0.199	0.053
WT	66/71 (93.0%)	27/34 (79.4%)		
Mut	5/71 (7.0%)	7/34 (20.6%)		

WT, wild type; Mut, mutant.

Table S12 The relationship between individual genes and pT stage

	pT stage		Phi coefficient	Fisher's P-value
	T1/T2 (n=48)	T3/T4 (n=57)		
BAP1			0.233	0.02
WT	47/48 (97.9%)	48/57 (84.2%)		
Mut	1/48 (2.1%)	9/57 (15.8%)		
PARP1			0.205	0.061
WT	48/48 (100%)	52/57 (91.2%)		
Mut	0/48 (0%)	5/57 (8.8%)		

WT, wild type; Mut, mutant.

Table S13 The relationship between individual genes and sarcomatoid

	Sarcomatoid		Phi coefficient	Fisher's P-value
	No (n=95)	Yes (n=10)		
BAP1			0.226	0.053
WT	88/95 (92.6%)	7/10 (70.0%)		
Mut	7/95 (7.4%)	3/10 (30.0%)		
MET			0.207	0.069
WT	87/95 (91.6%)	7/10 (70.0%)		
Mut	8/95 (8.4%)	3/10 (30.0%)		

WT, wild type; Mut, mutant.

Table S14 The relationship between individual genes and clinical stage

	Clinical stage		Phi coefficient	Fisher's P-value
	I/II (n=47)	III/IV (n=58)		
BAP1			0.227	0.022
WT	46/47 (97.9%)	49/58 (84.5%)		
Mut	1/47 (2.1%)	9/58 (15.5%)		

WT, wild type; Mut, mutant.

Table S15 The relationship between individual genes and ages

	Age		Phi coefficient	Fisher's P-value
	≥58 (n=58)	<58 (n=47)		
CCND1			0.247	0.021
WT	57/58 (98.3%)	40/47 (85.1%)		
Mut	1/58 (1.7%)	7/47 (14.9%)		

WT, wild type; Mut, mutant.

**Table S16** The relationship between individual genes and tumor thrombus

	Tumor thrombus		Phi coefficient	Fisher's P-value
	No (n=80)	Yes (n=25)		
PTEN			0.239	0.041
WT	79/80 (98.8%)	22/25 (88.0%)		
Mut	1/80 (1.2%)	3/25 (12.0%)		

WT, wild type; Mut, mutant.

Table S17 The relationship between individual genes and necrosis

|	Necrosis		Phi coefficient	Fisher's P value
	No (n=79)	Yes (n=26)		
BTG1			-0.196	0.062
WT	68/79 (86.1%)	26/26 (100%)		
Mut	11/79 (13.9%)	0/26 (0%)		

WT, wild type; Mut, mutant.
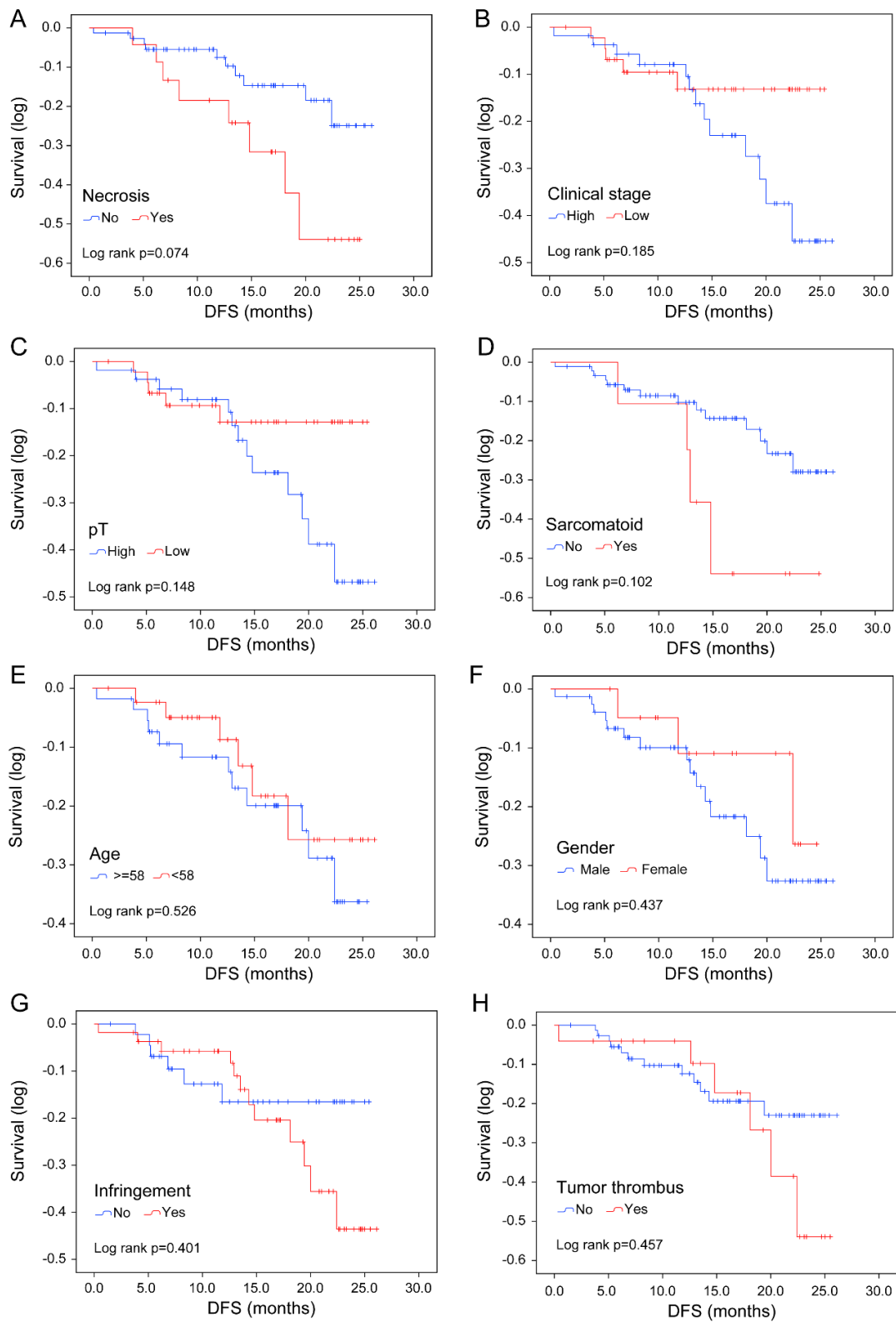
Figure S1 Kaplan-Meier curves for DFS of Chinese ccRCC patients with different clinical features. DFS, disease-free survival; ccRCC, clear cell renal cell carcinoma.
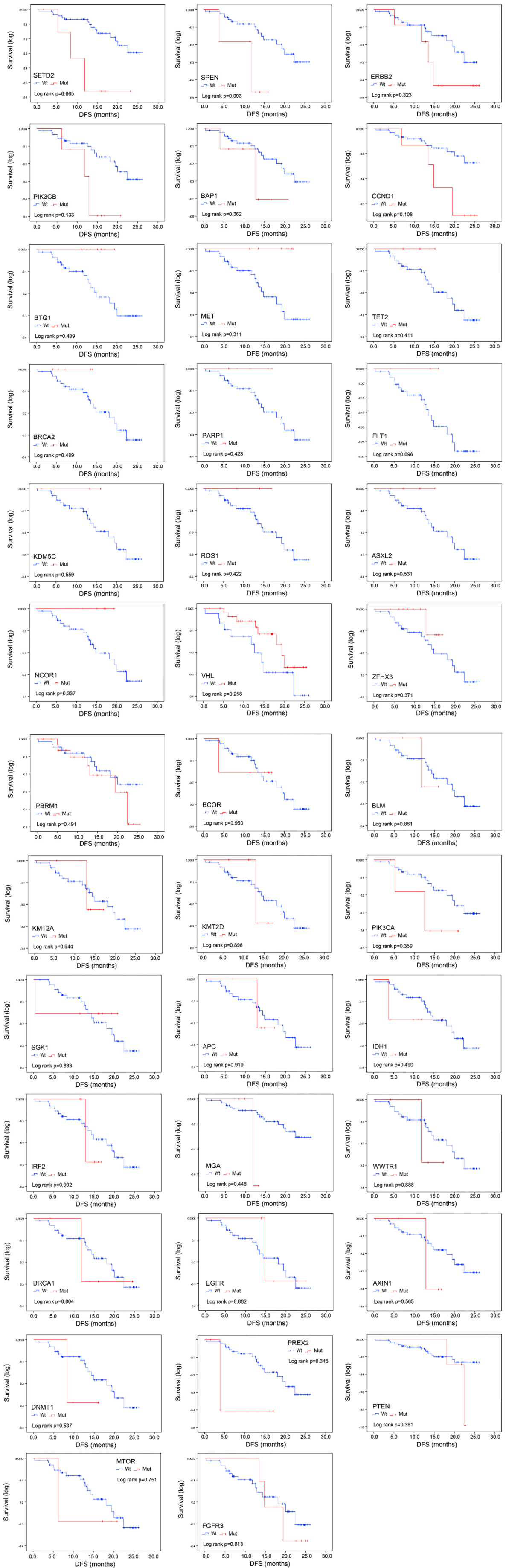
Figure S2 Kaplan-Meier curves for DFS of Chinese ccRCC patients with each mutated gene. DFS, disease-free survival; ccRCC, clear cell renal cell carcinoma.

Table S18 The mutations of genes associated with prognosis of Chinese ccRCC

Genes	Mutations
BAP1	p.S460fs, p.R179W, p.C320fs, p.S460*, p.Q684*, p.L361fs, p.S705fs, p.G194R, p.K205_A206deli nsS, p.A92V
PTEN	p.Y76Mfs*23, p.P246L, p.A151*fs*1, p.L112P, p.L182*
ERBB2	p.A241V, p.V1085L, CNV amplification
TP53	p.R175H, p.R273H, p.G325lfs*10, p.H168Lfs*5
CDK8	p.Q377del
TSC1	p.R500*, p.Q527*, p.W1063G, p.R509Q
SETD2	p.Q2484*, p.G704R, p.K568fs, p.W2395*, p.Y480*, p.K568*, p.N1289fs, p.Thr305fs, p.N801Tfs*17
SPEN	p.D3010Y, p.H1165L, p.P2465L, p.E1412K
BTG1	p.G64R

Altered in 94 (27.98%) of 336 samples.
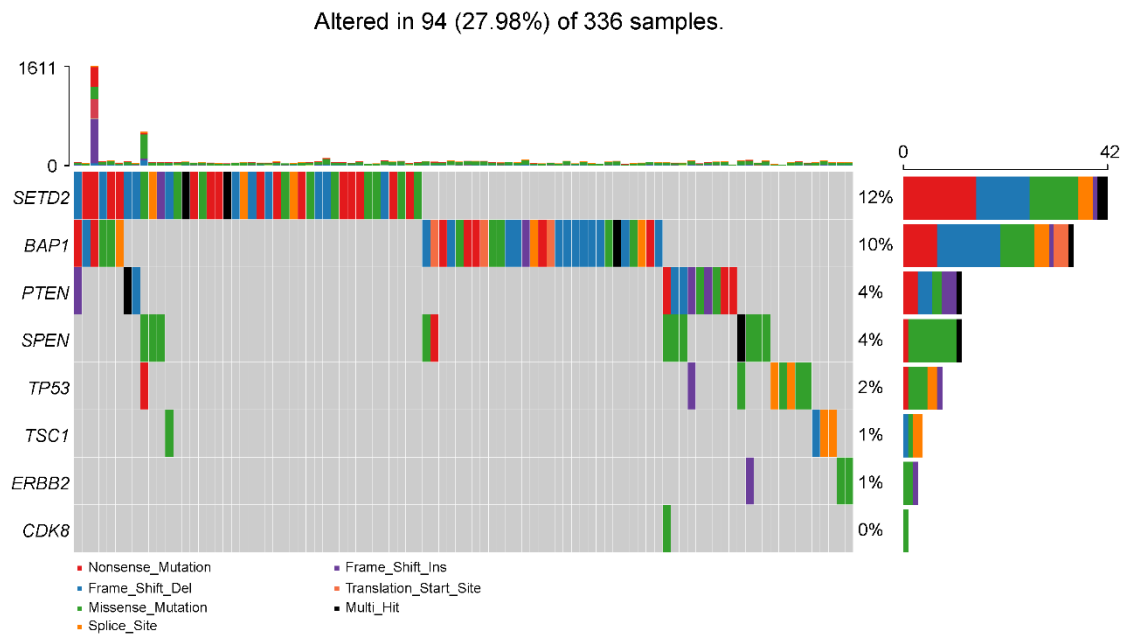
Figure S3 The mutation frequencies of the eight genes associated with poor prognosis in TCGA cohort. TCGA, The Cancer Genome Atlas.
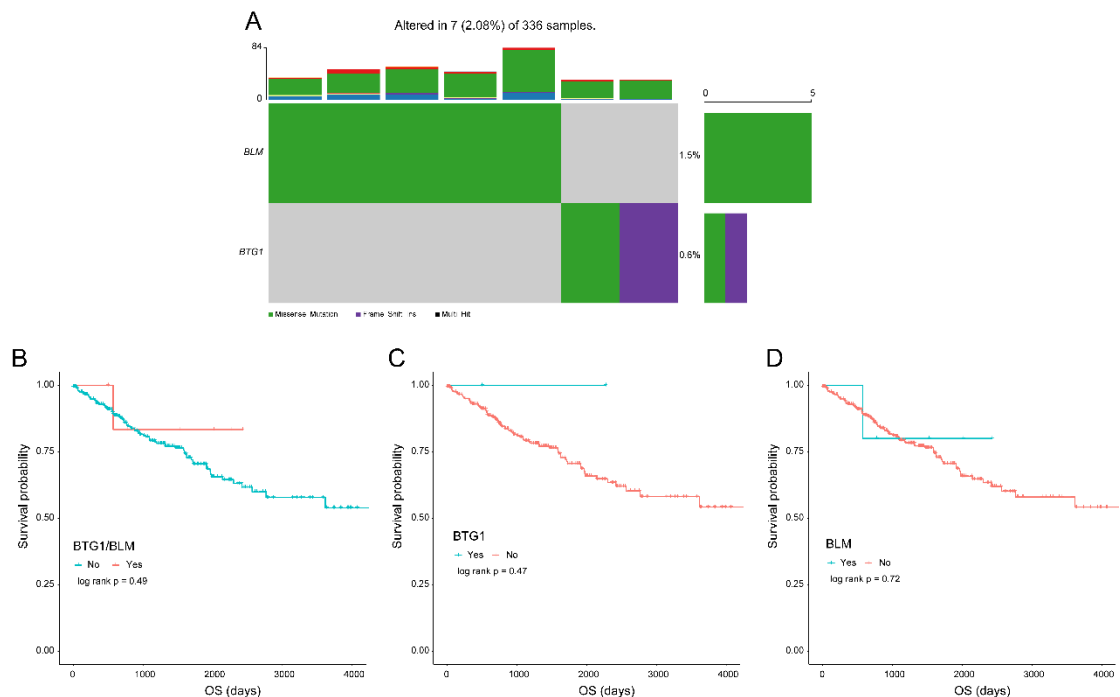
Figure S4 Validation of two genes (*BTG1*, *BLM*) associated with good prognosis using TCGA cohort. (A) The mutation frequencies of *BTG1* and *BLM* in TCGA cohort. (B) Kaplan-Meier curves for OS of ccRCC patients in TCGA cohort with mutated *BTG1/BLM*, (C) mutated *BTG1*, (D) mutated *BLM*. TCGA, The Cancer Genome Atlas; OS, overall survival; ccRCC, clear cell renal cell carcinoma
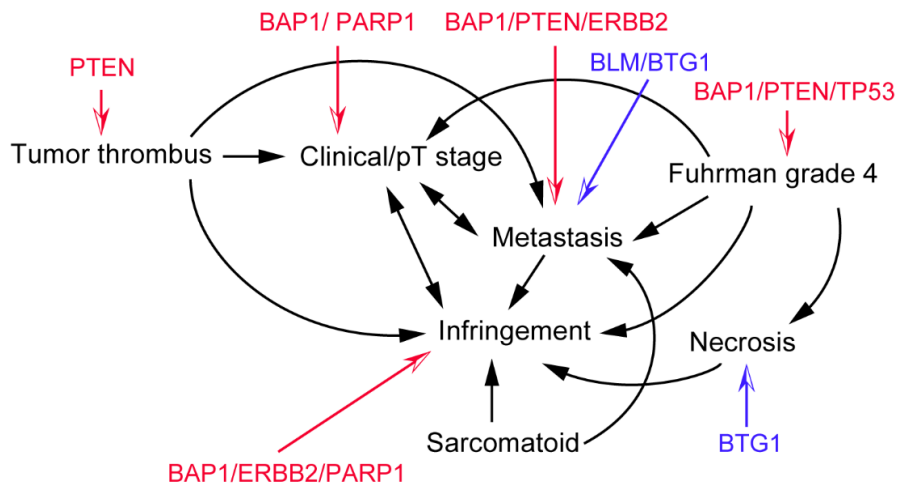
Figure S5 The interactions of different clinical indices. Genes associated with each clinical index were shown. Red, positively correlated; blue, negatively correlated.
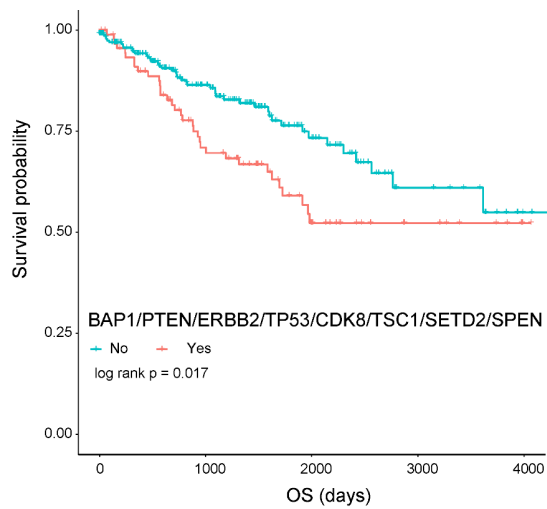
Figure S6 Kaplan-Meier curve for OS of ccRCC patients in TCGA cohort with 8 mutated genes associated with poor prognosis. TCGA, The Cancer Genome Atlas; OS, overall survival; ccRCC, clear cell renal cell carcinoma.