Characterization and Impact of *TERT* Promoter Region Mutations on Clinical Outcome in Renal Cell Carcinoma

Jozefina Casuscelli[a,b], **Maria F. Becerra**[c], **Brandon J. Manley**[c], **Emily C. Zabor**[d], **Ed Reznik**[e], **Almedina Redzematovic**[f], **Maria E. Arcila**[g], **Daniel M. Tennenbaum**[a], **Mazyar Ghanaat**[c], **Mahyar Kashan**[c], **Christian G. Stief**[b], **Maria Carlo**[f], **Martin H. Voss**[f], **Darren R. Feldman**[f], **Robert J. Motzer**[f], **Yingbei Chen**[g], **Victor E. Reuter**[g], **Jonathan A. Coleman**[c], **Paul Russo**[c], **James J. Hsieh**[a,f,†], **A. Ari Hakimi**[c,†,*]

[a]Human Oncology & Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[b]Department of Urology, Ludwig-Maximilians University, Munich, Germany

[c]Urology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[d]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[e]Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[f]Genitourinary Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[g]Department of Pathology Memorial Sloan Kettering Cancer Center, New York, NY, USA

[*]Corresponding author. Urology Service, Department of Surgery, Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan Kettering Cancer Center, 353 East 68th Street, New York, NY 10065, USA. Tel. +1 646 4224497; Fax: +1 212 9880760. hakimia@mskcc.org (A.A. Hakimi).
[†]These authors jointly directed this work.

Abstract

Background—Mutations in the promoter region of the *TERT* gene have been detected in a variety of cancers. These mutations can potentially lead to unlimited cell divisions and result in poor clinical prognosis.

Objective—To determine the role and relevance of *TERT* promoter region mutations in both clear cell (ccRCC) and non–clear cell (nccRCC) renal cell carcinoma using ultra-deep and whole-genome sequencing methods on primary tumor samples.

Design, setting, and participants—DNA from 281 kidney tumors (147 ccRCC and 134 nccRCC) was sequenced between 2013 and 2015, and clinical outcomes for these patients from a single institution were retrospectively analyzed.

Outcome measurements and statistical analysis—Differences in patient characteristics and mutational status were tested using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. Survival times were estimated using the Kaplan-Meier method and differences were tested using the log-rank test.

Results and limitations—*TERT* mutations occurred in 12.2% of ccRCC and 10.4% of nccRCC cases. In >80% of the cases, mutations were located at C228T and were found to co-occur only rarely with other relevant RCC driver genes. The median follow-up among survivors overall was 2.5 yr (range 0.1–18.3). *TERT* promoter mutations were significantly associated with cancer-specific survival in ccRCC (hazard ratio 2.68, 95% confidence interval 1.19–6.01; p = 0.013). In nccRCC, *TERT* mutations were significantly associated with larger tumors and metastatic development. Assessment of further relevant clinical associations was precluded in the nccRCC group by the heterogeneous and small sample size.

Conclusions—Our data suggests that *TERT* mutational status reflects a distinct pathogenesis with an aggressive disease course in RCC. Stratifying patients with this unique tumorigenesis that leads to poor clinical outcomes could be a putative target for novel therapeutics.

Patient summary—We show a previously unrecognized frequency of *TERT* promoter mutations in both clear cell and non–clear cell renal cell carcinoma. *TERT* promoter mutations were associated with some worse outcomes in patients with clear cell renal cell carcinoma.

Keywords

Renal cell carcinoma; Sequencing analysis; TERT promoter; Mortality; Prognosis

1. Introduction

Renal cell carcinoma (RCC) represents a heterogeneous group of malignancies comprising multiple histopathologic entities associated with a different landscape of molecular alterations in each subtype. Clear cell RCC (ccRCC) is the most common subtype, accounting for approximately 75% of all RCCs. The remaining subtypes are often grouped together as non–clear cell RCC (nccRCC), consisting of papillary RCC (15%), chromophobe RCC (5–10%), unclassified RCC (5%), and other rare entities [1].

Despite advances in our understanding of the genomic underpinnings of RCC, the full spectrum of cancer drivers in different histological subgroups is not yet completely understood. We sought to characterize the prevalence of further relevant somatic mutations in RCC, and focused specifically on the *TERT* gene, located on chromosome 5p. Up to 90% of human cancers have high levels of *TERT* RNA expression, and consequently high TERT enzyme activity. The TERT enzyme is responsible for addition of telomeric repeats to chromosomal ends [2]. Telomeres, located at the end of every chromosome, function to protect chromosomes from recombination and degradation. Telomeres are gradually shortened after every cell division, which leads to senescence and apoptosis of cells after a given number of divisions [3]. Telomerases, with their core unit TERT, are expressed and active in proliferating cells, and are downregulated in differentiated cells [4,5] because of transcriptional silencing [2].

The role of TERT in telomere and telomerase dynamics is the basis behind its oncogenic potential, whereny overactive *TERT* can maintain telomere length, enabling malignant cells to divide indefinitely and resulting in immortalization of cells [4,6]. Cancer-specific *TERT* transcription is putatively caused by mutations upstream of the translational start site of *TERT*, in the *TERT* promoter region [7]. Mutations in this crucial regulatory element allow *TERT* expression, leading to the creation of a novel binding site for the ETS family of transcription factors, which ultimately increases *TERT* transcriptional activity.

Overexpression of the *TERT* gene, and consequently high TERT activity, has been described in many cancers including melanoma, glioblastoma, and thyroid and urothelial tumors [8–12], and *TERT* promoter region mutations have been associated with advanced disease course and poor outcome [9,13–15].

It has previously been reported that the mutational status of the *TERT* promoter in RCC is only 6–10% [7,16–18]. However, most groups studied only ccRCC and the results were limited by the detection methods used, namely polymerase chain reaction and Sanger sequencing [17], which may underestimate the prevalence of *TERT* promoter alterations. Furthermore, the largest genomic characterization of ccRCC, performed by the Cancer Genome Atlas network [19], did not include the *TERT* gene in their analysis. The only comprehensive study that used next-generation sequencing to detect *TERT* promoter and gene aberrations investigated chromophobe RCC [18]. While *TERT* promoter mutations were detected in <5% of the cases analyzed, a wide range of breakpoints were observed within the *TERT* promoter region and were associated with *TERT* upregulation; however, the association with clinical outcomes was not established.

The aim of this study was to use whole-genome and ultra-deep target sequencing methods to characterize the prevalence of *TERT* gene and promoter mutations in RCC from a large single-institutional cohort. In addition, we aimed to define the impact of *TERT* mutations on clinical outcomes, namely metastatic development, recurrence, and disease-specific survival, in affected patients.

2. Patients and methods

2.1. Patient selection

After approval by the institutional review board (IRB #12-245, 89-076, and 06-107), our institutional kidney cancer database was queried to identify patients with RCC who underwent genomic testing of their renal tumors between 2013 and 2015. Tumor samples were retrieved either by surgical resection or biopsy performed between 1999 and 2015. All tumor samples included were reviewed and classified by expert genitourinary pathologists to select areas of maximum tumor content for DNA extraction. Individuals with sequencing results from metastatic tissue were excluded from the study. Primary tumor samples from a total of 281 patients were included in the analysis (Supplementary Fig. 1).

2.2. Next-generation sequencing

All patients had previously provided consent for sequencing purposes. DNA from primary tumors and matched normal tissue was extracted according to a standard protocol and subjected to analysis on two next-generation sequencing platforms. Germline mutations were ruled out by analysis of normal blood or adjacent nontumoral tissue for every sample. In our cohort, samples from 270 (96%) patients were sequenced using the MSK-IMPACT assay [20], a hybridization capture-based next-generation sequencing assay for targeted deep sequencing (approx. 500×) of all exons and selected introns of 341 or 410 oncogenes, tumor suppressor genes, and members of pathways deemed actionable by targeted therapies. Details on the MSK-IMPACT panel of targeted genes are provided in Supplementary Table 5.

The remaining 11 (3.9%) samples were subjected to whole-genome sequencing in collaboration with the New York Genome Center on an Illumina HiSeq 2500 system to a mean haploid depth coverage of 87× and 91×.

2.3. Statistical analysis

A panel of nine genes (*VHL, PBRM1, BAP1, SETD2, TERT, KDM5C, TP53, mTOR, PTEN*) was selected a priori for analysis on the basis of previously published data for known driver mutations in RCC [18,21–23]. Patient and disease characteristics were summarized using the median for continuous and the number (percentage) for categorical variables. Differences in patient and disease characteristics by histology were assessed using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. Associations between gene mutations and *TERT* promoter mutations were assessed using Fisher's exact test.

Cancer-specific survival (CSS) was evaluated for 279 patients with sufficient follow-up data. Recurrence-free survival (RFS) was evaluated for 185 patients who did not present with metastatic disease at the time of diagnosis. Follow-up time was calculated from the date of nephrectomy. Recurrence was defined as the date of pathologic confirmation of diagnosis. For patients with no pathological diagnosis, the date of the radiological examination was used instead. Patients with recurrence or stage IV disease and reported as deceased on last follow-up were considered to have died from disease. All analyses were stratified by

histology. CSS and RFS were estimated using the Kaplan-Meier method. Between-group comparisons were carried out using the log-rank test. Univariable Cox regression was used to evaluate associations of patient and disease characteristics with CSS and RFS. Similarly, univariable Cox regression was used to evaluate associations between gene mutations and CSS and RFS. For the ccRCC cohort we investigated multivariable models adjusted for SSIGN score (stage, size, grade, and necrosis) [24] to assess the association between *TERT* promoter mutations and CSS or RFS.

Statistical significance was set at $p < 0.05$. All statistical analyses were conducted using R software version 3.2.5 (R Core Development Team, Vienna, Austria).

3. Results

3.1. Overview

A total of 281 patients were analyzed, 147 (52.3%) with ccRCC and 134 (47.7%) with nccRCC. Baseline characteristics are listed in Table 1. The median patient age was 56.6 yr and 65.8% were male. ccRCC patients were older ($p = 0.011$), had higher grade ($p = 0.001$) and American Joint Committee on Cancer stage ($p = 0.004$) disease, and had a higher number of tumors with sarcomatoid features ($p = 0.001$) when compared to nccRCC patients. The study populations differ significantly in terms of demographics, grade and stage, and sarcomatoid features, so we stratified our analyses by ccRCC and nccRCC. Gene mutation frequencies for the nine genes selected (*VHL, PBRM1, BAP1, SETD2, TERT, KDM5C, TP53, mTOR, PTEN*) and the presence or absence or metastases at last follow-up are described in Figure 1A and Figure 1A for the ccRCC and the nccRCC groups, respectively. In 81(29%) patients, no genomic alterations in the panel studied were detected. Further mutations assessed with target or whole-genome sequencing did not reach frequencies >2%.

TERT mutations were present in 12.2% (18/147) of ccRCC and 10.4% (14/134) of nccRCC cases. Of these alterations, the promoter region was affected in 17/18 (94.4%) ccRCC and 13/14 (92.8%) nccRCC patients.

3.2. ccRCC cohort

The genes most commonly mutated in the 147 ccRCC patients were located on chromosome 3p: *VHL* (81.0%), *PBRM1* (42.9%), *SETD2* (27.9%), and *BAP1* (20.4%). *TERT* alterations were the fifth most frequently assessed mutations, at 12.2% ($n = 18$; Fig. 1A). Among those, 17 patients (94.4%) had alterations detected in the promoter region, and one (5.6%) was diagnosed with a *TERT* gene mutation and was excluded from further association analyses. *TERT* promoter alterations were mutually exclusive in all cases: 13 (76.5%) were at site C228T, two (11.8%) at C250T, and two (11.8%) at C898T.

VHL mutations co-occurred in 70.6% of the cases with *TERT* promoter mutations. Among patients with *TERT* promoter mutations, 88.2% were wild-type for *PBRM1* ($p = 0.008$). *TERT* promoter mutations were not significantly associated with any of the other mutations studied (Fig. 1A and Supplementary Table 1). Nevertheless, the low mutation frequencies might have low power for detecting associations.

A total of 54 patients with metastatic disease at baseline and six patients who did not have surgery were excluded from the RFS analysis. The median follow-up for ccRCC patients was 3.1 yr for those who did not die from cancer and 3.2 yr for patients without recurrence. During follow-up, 32 patients died from RCC and 42 patients experienced disease recurrence. Univariable Cox regression results for the association with CSS and RFS are presented in Table 2. Higher tumor size and grade, sarcomatoid status, and SSIGN score [24] were significantly associated with higher risks of disease recurrence and death from cancer (Table 2). The survival analysis showed that patients with *TERT* promoter mutations had significantly shorter CSS (hazard ratio [HR] 2.68, 95% confidence interval [CI] 1.19–6.01; $p = 0.013$; Fig. 2A). However, there was no significant association between *TERT* promoter mutations and RFS (HR 1.35, 95% CI 0.48–3.79; $p = 0.567$; Fig. 2B). Analysis of associations between other mutations and CSS and RFS revealed that *BAP1* mutations were associated with a higher risk of cancer-specific death, whereas *SETD2* mutations were associated with a risk of tumor recurrence (Table 3).

Multivariable Cox regression models were adjusted for SSIGN score to assess the association between mutations and CSS and RFS (Supplementary Table 2). *TERT* promoter (HR 3.55, 95% CI 1.37–9.20; $p = 0.009$) and *BAP1* mutations (HR 2.93, 95% CI 1.28–6.71; $p = 0.011$) were significantly associated with higher risk of cancer-specific death. Only *TP53* mutations were significantly associated with higher risk of recurrence (HR 2.17, 95% CI 1.07–4.41; $p = 0.033$).

3.3. nccRCC cohort

A total of 134 tumors, consisting of a variety of tumor histologies (Supplementary Table 3), were analyzed in the nccRCC cohort. The genes most frequently mutated among those evaluated for analysis were *TP53* (25.4%), *TERT* (10.4%), *PTEN* (8.2%), and *mTOR* (6.7%; (Fig. 1B). A *TERT* gene mutation was observed for one patient (E441del). All 13 non-exonic alterations affected the promoter region, of which 11 alterations involved hotspot C228T (84.6%).

TERT promoter mutations showed no association with the incidence of mutations in the other genes evaluated. Moreover, they were mutually exclusive to mutations in all the other genes, except for *TP53* in two cases and *SETD2* in one case (Fig. 2B and Supplementary Table 4).

Univariable Cox regression analyses were performed to test the association of patient and disease characteristics with CSS and RFS (Table 4). Two patients were excluded from the CSS analysis because of unknown cause of death. A total of 33 patients with metastatic disease at presentation and three patients who did not undergo surgery were excluded from the RFS analysis. The median follow-up among nccRCC patients was 2.2 yr for those who did not die from cancer and 2.3 yr for patients who did not experience recurrence. During follow-up, 38 patients died from RCC and 51 patients had disease recurrence.

Sarcomatoid differentiation was significantly association with higher risk of death from cancer ($p = 0.004$); no factors were significantly associated with RFS (Table 4).

Survival analysis for patients with and without *TERT* promoter mutations revealed a wild-type *TERT* advantage for CSS, but statistical significance was not reached (Fig. 3).

4. Discussion

The role of *TERT* in human mutagenesis was initially described in melanoma [10]. Subsequent studies showed that *TERT* promoter mutations are associated with worse survival outcomes in a variety of cancer types [25]. Our results show that *TERT* promoter mutations are common in ccRCC and are associated with poor clinical outcomes. As previously described, *TERT* promoter mutations were commonly detected in chromophobe RCC, but they also occurred in other nccRCC subtypes (papillary, unclassified and TFE3 translocation RCC).

Univariable analyses confirmed that *TERT* variations were associated with shorter CSS in only ccRCC. The lack of association in the nccRCC group may be due to the heterogeneous nature of the cohort.

The known hotspot mutation C228T was the most commonly mutated site in ~80% of our cases, followed by C250T. Previous studies have shown that these hotspot mutations lead to higher *TERT* expression and subsequent telomerase activity, which is ultimately associated with cancer progression and poor prognosis [26].

TERT promoter mutations allow the creation of ETS1 binding sites, leading to TERT transcription [27]. Recent data suggest that ETS transcription factors participate in the upregulation of hypoxia-inducible genes that are a hallmark of cells with defective *VHL* complex, as in ccRCC [28]. Furthermore, it has been shown that hypoxia increases ETS1 transcription and transactivation [29], which points to a cooperative effect with HIF. This supports the observation that TERT expression is enhanced in cases of concomitant *VHL* gene inactivation and *TERT* promoter mutation [16]. In our ccRCC cohort, we found that *VHL* mutations occurred in approximately 70% of patients carrying *TERT* promoter mutations. However, further co-occurrences could not be detected in ccRCC, possibly indicating the involvement of specific oncogenic pathways in tumors with *TERT* promoter mutations.

TERT promoter mutations were mutually exclusive to most genes tested in nccRCC. This suggests that *TERT* mutations are responsible for a unique pathway that leads to aggressive tumor behavior; however, the heterogeneous and incompletely unraveled genomic landscape of these entities poses difficulties in the identification of clear genomic drivers for cancer progression.

Studies in bladder cancer with suppression of high *TERT* expression and telomerase activity showed promising results that may lead to treatments targeting tumors bearing *TERT* alterations [30]. We therefore believe that identifying *TERT* promoter alterations, as one of the most commonly mutated sites in kidney cancer, may reveal a valuable actionable target, in particular for patients lacking other alterations.

Our study has multiple limitations. Our cohort consisted of a high-risk population of patients treated at a tertiary referral center, which does not reflect the spectrum of RCC and potentially over-represents aggressive tumors bearing *TERT* promoter mutations. Furthermore, our cohort does not reflect the common histological landscape of nccRCC given the over-representation of chromophobe RCC, and may mask a higher incidence of *TERT* mutations in papillary RCC. In addition, systemic therapy data were not available. Larger validation studies with longer follow-up are needed to evaluate the impact of *TERT* promoter mutations in kidney cancer. Finally, tumor heterogeneity is a crucial factor that needs to be considered when assessing mutational status. Therefore, future analyses of multiple regions from primary tumors and matched metastatic sites are necessary to determine the relevance of *TERT* promoter mutations in RCC. Nevertheless, our findings are significant and help to expand knowledge of the genomic drivers of RCC.

5. Conclusions

Our data suggest that *TERT* promoter mutations are associated with worse CSS in ccRCC. Our work has highlighted a previously unrecognized frequency of *TERT* promoter mutations in both clear cell and non–clear cell RCC. The patterns of mutual exclusivity with other known cancer drivers in nccRCC suggest a unique tumorigenesis pathway in patients bearing *TERT* promoter mutations. Further studies are needed to validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Srigley JR, Delahunt B, Eble JN, et al. The International Society of Urological Pathology (ISUP) Vancouver classification of renal neoplasia. Am J Surg Pathol. 2013; 37:1469–89. [PubMed: 24025519]

2. Aubert G. Telomere dynamics and aging. Nat Genet. 2014; 125:89–111.

3. Counter CM, Avilion AA, LeFeuvre CE, et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J. 1992; 11:1921–9. [PubMed: 1582420]

4. Chiba K, Johnson JZ, Vogan JM, Wagner T, Boyle JM. Cancer-associated TERT promoter mutations abrogate telomerase silencing. eLife. 2015; 4:e07198.

5. Bell RJ, Rube HT, Xavier-Magalhaes A, et al. Understanding TERT promoter mutations: a common path to immortality. Mol Cancer Res. 2016; 14:315–23. [PubMed: 26941407]

6. Greider CW, Blackburn EH. Tracking telomerase. Cell. 2004; 116(2 Suppl):S83–86. [PubMed: 15055591]

7. Vinagre J, Almeida A, Populo H, et al. Frequency of TERT promoter mutations in human cancers. Nat Commun. 2013; 4:2185. [PubMed: 23887589]

8. Jin L, Chen E, Dong S, et al. BRAF and TERT promoter mutations in the aggressiveness of papillary thyroid carcinoma: a study of 653 patients. Oncotarget. 2016; 7:18346–55. [PubMed: 26943032]

9. Simon M, Hosen I, Gousias K, et al. TERT promoter mutations: a novel independent prognostic factor in primary glioblastomas. Neuro-oncology. 2015; 17:45–52. [PubMed: 25140036]

10. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013; 339:957–9. [PubMed: 23348506]

11. Cowan M, Springer S, Nguyen D, et al. High prevalence of TERT promoter mutations in primary squamous cell carcinoma of the urinary bladder. Mod Pathol. 2016; 29:511–5. [PubMed: 26965579]

12. Borah S, Xi L, Zaug AJ, et al. Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. Science. 2015; 347:1006–10. [PubMed: 25722414]

13. Heidenreich B, Rachakonda PS, Hosen I, et al. TERT promoter mutations and telomere length in adult malignant gliomas and recurrences. Oncotarget. 2015; 6:10617–33. [PubMed: 25797251]

14. Rachakonda PS, Hosen I, de Verdier PJ, et al. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proc Natl Acad Sci U S A. 2013; 110:17426–31. [PubMed: 24101484]

15. Huang DS, Wang Z, He XJ, et al. Recurrent TERT promoter mutations identified in a large-scale study of multiple tumour types are associated with increased TERT expression and telomerase activation. Eur J Cancer. 2015; 51:969–76. [PubMed: 25843513]

16. Wang K, Liu T, Liu L, et al. TERT promoter mutations in renal cell carcinomas and upper tract urothelial carcinomas. Oncotarget. 2014; 5:1829–36. [PubMed: 24742867]

17. Hosen I, Rachakonda PS, Heidenreich B, et al. TERT promoter mutations in clear cell renal cell carcinoma. Int J Cancer. 2015; 136:2448–52. [PubMed: 25331263]

18. Davis CF, Ricketts CJ, Wang M, et al. The somatic genomic landscape of chromophobe renal cell carcinoma. Cancer Cell. 2014; 26:319–30. [PubMed: 25155756]

19. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013; 499:43–9. [PubMed: 23792563]

20. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn. 2015; 17:251–64. [PubMed: 25801821]

21. De Velasco G, Miao DD, Voss MH, et al. Tumor mutational load and immune parameters across metastatic renal cell carcinoma (mRCC) risk groups. Cancer Immunol Res. 2016; 4:820–2. [PubMed: 27538576]

22. Hakimi AA, Mano R, Ciriello G, et al. Impact of recurrent copy number alterations and cancer gene mutations on the predictive accuracy of prognostic models in clear cell renal cell carcinoma. J Urol. 2014; 192:24–9. [PubMed: 24518768]

23. Hakimi AA, Ostrovnaya I, Reva B, et al. Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: a report by MSKCC and the KIRC TCGA research network. Clin Cancer Res. 2013; 19:3259–67. [PubMed: 23620406]

24. Frank I, Blute ML, Cheville JC, Lohse CM, Weaver AL, Zincke H. An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy based on tumor stage, size, grade and necrosis: the SSIGN score. J Urol. 2002; 168:2395–400. [PubMed: 12441925]

25. Yuan P, Cao JL, Abuduwufuer A, et al. Clinical characteristics and prognostic significance of TERT promoter mutations in cancer: a cohort study and a meta-analysis. PLoS One. 2016; 11:e0146803. [PubMed: 26799744]

26. Huang FW, Bielski CM, Rinne ML, et al. TERT promoter mutations and monoallelic activation of TERT in cancer. Oncogenesis. 2015; 4:e176.

27. Vallarelli AF, Rachakonda PS, Andre J, et al. TERT promoter mutations in melanoma render TERT expression dependent on MAPK pathway activation. Oncotarget. 2016; 7:53127–36. [PubMed: 27449293]

28. Salnikow K, Aprelikova O, Ivanov S, et al. Regulation of hypoxia-inducible genes by ETS1 transcription factor. Carcinogenesis. 2008; 29:1493–9. [PubMed: 18381358]

29. Qiao N, Xu C, Zhu YX, Cao Y, Liu DC, Han X. Ets-1 as an early response gene against hypoxia-induced apoptosis in pancreatic beta-cells. Cell Death Dis. 2015; 6:e1650. [PubMed: 25695603]

30. Li C, Wu S, Wang H, et al. The C228T mutation of TERT promoter frequently occurs in bladder cancer stem cells and contributes to tumorigenesis of bladder cancer. Oncotarget. 2015; 6:19542–51. [PubMed: 26143634]

Take Home Message

TERT mutational status reflects a distinct pathogenesis with an aggressive disease course in clear cell renal cell carcinoma. Stratifying patients with this unique tumorigenesis that leads to poor clinical outcomes could be a putative target for novel therapeutics.
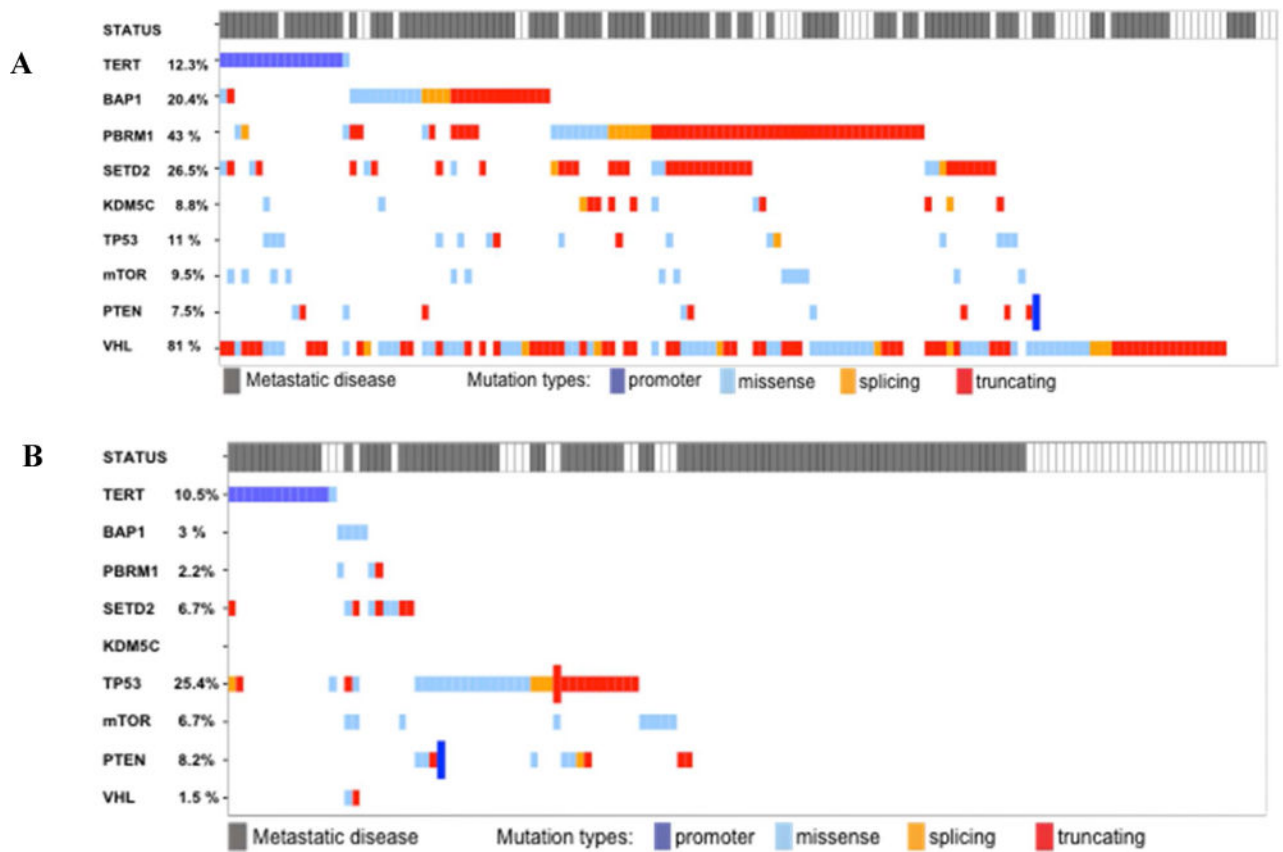
Fig. 1.
Overview of concordance and mutual exclusivity of *TERT* promoter mutations and nine selected known renal cell carcinoma (RCC) driver genes in (A) clear cell RCC and (B) non–clear cell RCC. The presence of metastatic disease at last follow-up is highlighted in grey in the top row.

Fig. 2.

Probability of (A) cancer-specific survival (CSS) and (B) recurrence-free survival (RFS) by *TERT* status among patients with clear cell renal cell carcinoma. The median follow-up was 3.1 yr (range 0.1–11.2) for CSS and 3.2 yr (range 0.1–11.2) for RFS. wt = wild type; mut = mutant.

Fig. 3.

Probability of (A) cancer-specific survival (CSS) and (B) recurrence-free survival (RFS) by *TERT* status among patients with non–clear cell re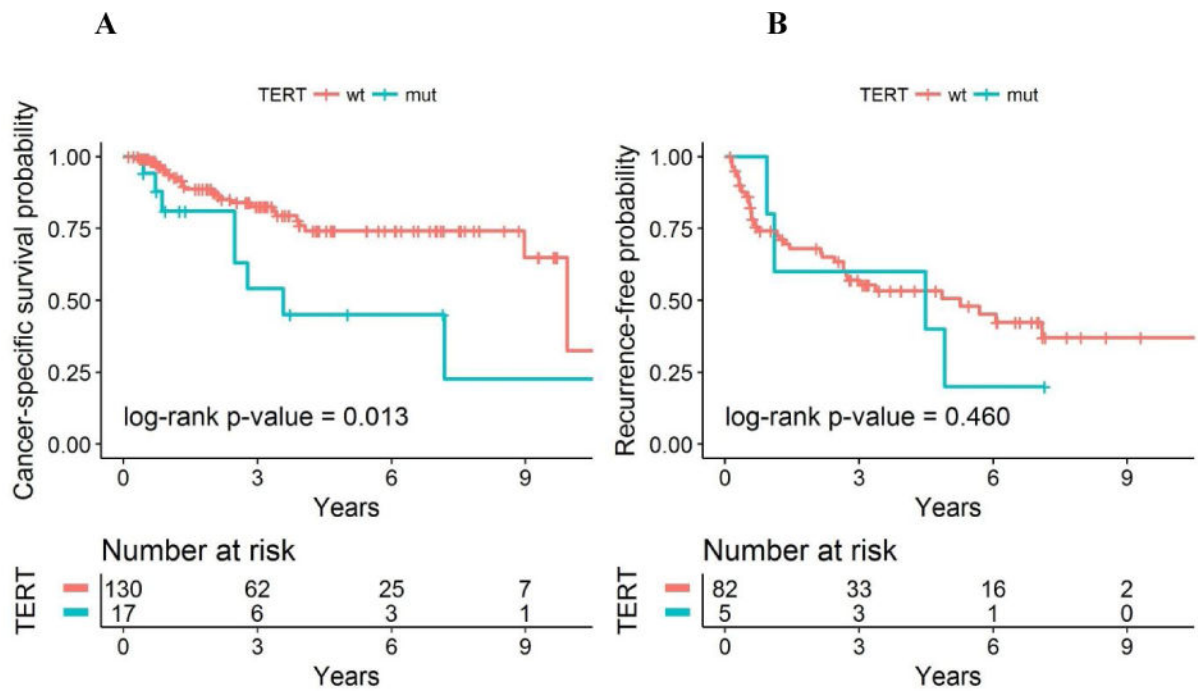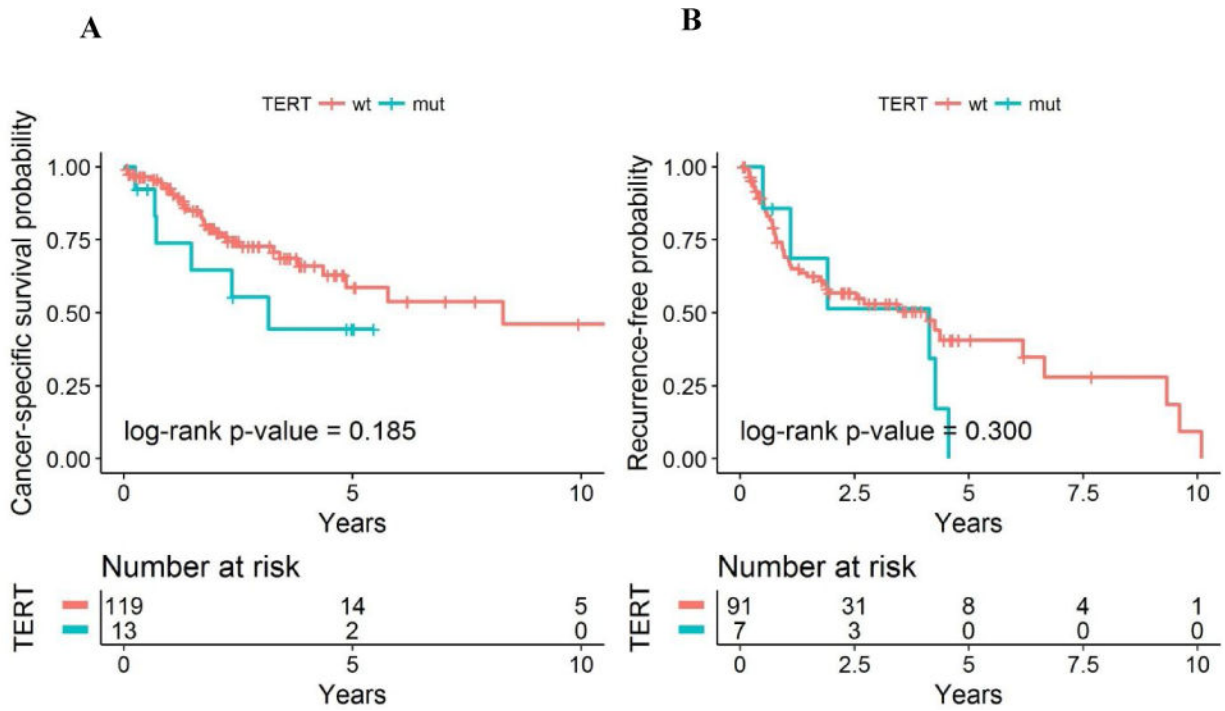nal cell carcinoma. The median follow-up was 2.2 yr (range 0.1–18.3) for CSS and 2.3 yr (range 0.1–7.7) for RFS. wt = wild type; mut = mutant.

Table 1

Demographic data and baseline clinical characteristics

	Overall	ccRCC (*n* = 147)	nccRCC (*n* = 134)	*p* value
Age, yr (range)	56.6 (19.1 – 86.3)	58.0 (31.2 – 81)	53.5 (19.1 – 86.3)	0.011
Tumor size, cm (range)	8.0 (1.2 – 29.0)	8.0 (2.0 – 22.2)	8.4 (1.2 – 29)	0.320
Gender, *n* (%)				0.315
Female	96 (34.2)	46 (31.3)	50 (37.3)	
Male	185 (65.8)	101 (68.7)	84 (62.7)	
Grade, *n* (%)				0.001
G2	24 (8.5)	22 (15.0)	2 (1.5)	
G3	96 (34.2)	61 (41.5)	35 (26.1)	
G4	73 (26.0)	62 (42.2)	11 (8.2)	
NA	88 (31.3)	2 (1.4)	86 (64.2)	
Sarcomatoid, *n* (%)				0.001
No	205 (73.0)	96 (65.3)	109 (81.3)	
Yes	70 (24.9)	49 (33.3)	21 (15.7)	
NA	6 (2.1)	2 (1.4)	4 (3.0)	
T stage, *n* (%)				0.007
T1	64 (22.7)	29 (19.8)	35 (26.1)	
T2	41 (14.6)	14 (9.6)	27 (20.2)	
T3	157 (55.8)	96 (65.3)	61 (45.6)	
T4	11 (3.9)	5 (3.4)	6 (4.5)	
NA	8 (2.8)	3(2.0)	5 (3.7)	
N stage, *n* (%)				0.271
N0	106 (37.7)	61 (41.5)	45 (33.6)	
N1	35 (12.5)	14 (9.5)	21 (15.7)	
N2	10 (3.6)	4 (2.7)	6 (4.5)	
Nx	122 (43.4)	65 (44.2)	57 (42.5)	
NA	8 (2.8)	3 (2.0)	5 (3.7)	
M stage, *n* (%)				<0.001
M0	193 (68.7)	93 (63.3)	100 (74.6)	
M1	87 (31)	54 (36.7)	33 (24.6)	
NA	1 (0.4)	0 (0)	1 (0.7)	
AJCC				0.004
I	52 (18.5)	22 (15.0)	30 (22.4)	
II	28 (10.0)	8 (5.4)	20 (14.9)	
III	105 (37.4)	56 (38.1)	49 (36.6)	
IV	96 (34.2)	61 (41.5)	35 (26.1)	

RCC = renal cell carcinoma; cc = clear cell; ncc = non–clear cell; NA = not available; AJCC = American Joint Committee on Cancer.

Table 2

Univariable Cox regression results for the association of patient and disease characteristics with cancer-specific and recurrence-free survival among patients with clear cell renal cell carcinoma

	Cancer - specific survival		Recurrence - free survival	
	HR (95% CI)	***p* value**	**HR (95% CI)**	***p* value**
Age	1.01 (0.97 – 1.04)	0.644	0.99 (0.96 – 1.02)	0.526
Tumor size (cm)	1.12 (1.05 – 1.20)	0.001	1.24 (1.13 – 1.36)	<0.001
Gender		0.388		0.892
Female	1		1	
Male	0.73 (0.36 – 1.49)		0.96 (0.51 – 1.81)	
Grade		<0.001		<0.001
G2	1		1	
G3	0.59 (0.10 – 3.54)		4.35 (1.29 – 14.69)	
G4	9.11 (2.14 – 38.9)		11.74 (3.41 – 40.45)	
Sarcomatoid		<0.001		0.011
No	1		1	
Yes	11.45 (4.89 – 26.78)		2.45 (1.23 – 4.9)	
SSIGN score	1.26 (1.14 – 1.40)	<0.001	1.16 (1.07 – 1.27)	0.001

HR = hazard ratio; CI = confidence interval.

Table 3

Univariable Cox regression results for the association of mutations with cancer-specific and recurrence-free survival among patients with clear cell renal cell carcinoma Wild-type (wt), Mutant (mut)

Gene	Cancer - specific survival		Recurrence - free survival	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
VHL		0.227		0.080
Wild type	1.00		1.00	
Mutant	0.59 (0.25 – 1.38)		0.53 (0.26 – 1.08)	
PBRM1		0.475		0.407
Wild type	1.00		1.00	
Mutant	0.77 (0.38 – 1.58)		0.77 (0.42 – 1.42)	
SETD2		0.453		0.001
Wild type	1.00		1.00	
Mutant	1.32 (0.64 – 2.73)		3.09 (1.63 – 5.86)	
BAP1		0.002		0.141
Wild type	1.00		1.00	
Mutant	3.10 (1.52 – 6.30)		1.74 (0.83 – 3.65)	
KDM5C		0.255		0.682
Wild type	1.00		1.00	
Mutant	0.31 (0.04 – 2.31)		1.28 (0.39 – 4.15)	
TP53		0.567		0.053
Wild type	1.00		1.00	
Mutant	0.66 (0.15 – 2.79)		2.37 (0.99 – 5.71)	
mTOR		0.701		0.34
Wild type	1.00		1.00	
Mutant	1.23 (0.42 – 3.58)		0.5 (0.12 – 2.07)	
PTEN		0.097		0.333
Wild type	1.00		1.00	
Mutant	2.25 (0.86 – 5.88)		1.66 (0.59 – 4.68)	

HR = hazard ratio; CI = confidence interval.

Table 4

Univariable Cox regression results for the association of patient and disease characteristics with cancer-specific and recurrence-free survival among patients with non–clear cell renal cell carcinoma patients

	Cancer - specific survival		Recurrence - free survival	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age	1 (0.98 – 1.03)	0.869	0.99 (0.97 – 1.01)	0.534
Tumor size (cm)	1.02 (0.96 – 1.09)	0.541	1.03 (0.99 – 1.08)	0.168
Gender		0.932		0.454
Female	1.00		1.00	
Male	1.03 (0.54 – 1.98)		0.82 (0.48 – 1.39)	
Sarcomatoid		0.004		0.035
No	1.00		1.00	
Yes	2.77 (1.38 – 5.55)		2.11 (1.05 – 4.23)	
TERT promoter		0.191		0.261
Wild type	1.00		1.00	
Mutant	1.8 (0.75 – 4.36)		1.58 (0.71 – 3.51)	

HR = hazard ratio; CI = confidence interval.