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Prognostic Value of *TERT* Alterations and Mutational and Copy Number Alteration Burden in Urothelial Carcinoma

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Abstract

Point mutations in the *TERT* gene promoter occur at high frequency in multiple cancers, including urothelial carcinoma (UC). However, the relationship between *TERT* promoter mutations and UC

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patient outcomes is unclear owing to conflicting reports in the literature. In this study we examined the association of *TERT* alterations, tumor mutational burden per megabase (Mb), and copy number alteration (CNA) burden with clinical parameters and their prognostic value in a cohort of 398 urothelial tumors. The majority of *TERT* mutations were located at two promoter region hotspots (chromosome 5, 1 295 228 C>T and 1 295 250 C>T). *TERT* alterations were more frequently present in bladder tumors than in upper tract tumors (73% vs 53%; $p = 0.001$). *ARID1A*, *PIK3CA*, *RB1*, *ERCC2*, *ERBB2*, *TSC1*, *CDKN1A*, *CDKN2A*, *CDKN2B*, and *PTPRD* alterations showed significant co-occurrence with *TERT* alterations (all $p < 0.0025$). *TERT* alterations and the mutational burden/Mb were independently associated with overall survival (hazard ratio[HR] 2.31, 95% confidence interval [CI] 1.46–3.65; $p < 0.001$; and HR 0.96, 95% CI 0.93–0.99; $p = 0.002$), disease-specific survival (HR 2.23, 95% CI 1.41–3.53; $p < 0.001$; and HR 0.96, 95% CI 0.93–0.99; $p = 0.002$), and metastasis-free survival (HR 1.63, 95% CI 1.05–2.53; $p = 0.029$; and HR 0.98, 95% CI 0.96–1.00; $p = 0.063$) in multivariate models.

Point mutations in the *TERT* gene promoter occur at high frequency in multiple cancers, including urothelial carcinoma (UC). These mutations create novel consensus binding sites for ETS family transcription factors, leading to upregulated telomerase expression. Borah et al [1] showed that *TERT* promoter mutations correlate with higher levels of *TERT* mRNA and protein expression, telomerase enzyme activity, and telomere length in a study of 23 human UC cell lines. However, the relationship between *TERT* promoter mutations and UC patient outcomes is unclear owing to conflicting reports in the literature [2,3]. Furthermore, the genomic characterization of bladder UC performed by The Cancer Genome Atlas (TCGA) using-whole exome sequencing did not include the *TERT* promoter region in their analysis [4]. We previously showed that a subset of bladder tumors have a high burden of copy number alterations (CNAs) using array comparative genomic hybridization [5]. However, the extent of the CNA burden did not predict survival in a cohort of 97 high-grade bladder tumors [5]. In addition, among tumor types subjected to TGCA analysis, bladder tumors have a relatively high mutational burden per megabase (Mb). Of note, it has been shown that a high mutational count is associated with better clinical outcome among UC patients treated with immunotherapy [6].

In this study, we examined the association between *TERT* alterations and tumor mutational and CNA burden with clinical parameters in a cohort of 398 urothelial tumors. Tumors were profiled using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) next-generation sequencing assay [7] in a clinical laboratory with Clinical Laboratory Improvement Amendments certification according to an institutional review board–approved prospective sequencing protocol. MSK-IMPACT uses paired tumor and germline DNA to identify somatic point mutations, insertions/deletions, CNAs, and select translocations in all exons and select introns for 341 oncogenes and tumor suppressor genes. Notably, MSK-IMPACT covers the entire *TERT* promoter region. CNA burden was defined as fraction of the tumor genome affected by CNAs.

Among the 398 UC patients analyzed, 286 mutations and seven *TERT* gene amplifications were identified in 276 tumors (Supplementary Table 1). The majority of the *TERT* mutations were localized to two promoter region hotspots (chromosome 5, 1 295 228 C>T and 1 295

250 C>T; Fig. 1A). The median allele frequency for *TERT* mutations was 32% (interquartile range 19–44%). Patients with no *TERT* mutation ($n = 122$), *TERT* promoter mutations ($n = 259$), *TERT* gene amplification ($n = 2$), and concomitant *TERT* promoter mutations and gene amplification ($n = 5$) were included in this analysis. We excluded cases with *TERT* mutations of unknown significance ($n = 10$) for the analysis. Of note, inclusion of cases with *TERT* mutations of unknown significance ($n = 10$) did not alter the results.

The demographic, clinical, and pathological characteristics of the patients in the cohort are summarized in Supplementary Table 2. Patients with *TERT* alterations (promoter mutations and/or amplification) were significantly older than those without *TERT* alterations (median age 67 vs 64 yr; $p = 0.03$). UC with *TERT* alterations had a significantly higher mutational burden/Mb (median 8 vs 4; $p < 0.001$) and a significantly higher CNA burden (median 0.12 vs 0.05; $p < 0.001$). *TERT* alterations were more frequently present in bladder UC than in upper tract UC (73% vs 53%; $p = 0.001$). There was no association between *TERT* alterations and tumor stage or tumor grade (Supplementary Table 2). Supplementary Figure 1 shows the association of mutational burden/Mb and CNA burden with tumor stage and tumor grade. There was a significant association between CNA burden and tumor stage ($p < 0.001$) and tumor grade ($p < 0.001$). Supplementary Figure 2 shows the relationship between mutational burden/Mb and CNA burden (Spearman's $\rho = 0.138$; $p = 0.006$).

We also examined whether *TERT* alterations co-occurred with or mutually exclusive from alterations in other known oncogenes and tumor suppressor genes. *ARID1A*, *PIK3CA*, *RB1*, *ERCC2*, *ERBB2*, *TSC1*, *CDKN1A*, *CDKN2A*, *CDKN2B*, and *PTPRD* alterations showed significant co-occurrence with *TERT* alterations after Bonferroni correction to adjust for multiple comparisons (all $p < 0.0025$; Fig. 1B and Supplementary Table 3). *TERT* and *ATRX* gene alterations were mutually exclusive, but this association was not significant after adjusting for multiple comparisons ($p = 0.02$). Notably, it has been shown that *TERT* alterations are mutually exclusive from *ATRX* alterations, as tumors with *ATRX* alterations utilize the telomerase-independent alternative lengthening of telomeres mechanism to maintain telomere length [8,9]. Heidenreich et al [10] reported that *TERT* alterations co-occurred with *CDKN2A* alterations. A few investigators [3] reported co-occurrence of *TERT* alterations and *FGFR3* alterations; however, we did not find a significant association between *FGFR3* and *TERT* alterations in our cohort.

Supplementary Table 4 shows the results of a univariate Cox regression analysis of the clinical and pathological parameters evaluated for overall survival (OS), disease-specific survival (DSS), and metastasis-free survival (MFS). *TERT* alterations and the mutational burden/Mb were independent predictors for OS ($p < 0.001$ and 0.002), DSS ($p < 0.001$ and 0.002), and MFS ($p = 0.029$ and 0.063) in multivariate Cox regression models (Table 1).

Tumors with *TERT* alterations had worse prognosis compared to tumors without *TERT* alterations, whereas tumors with a higher mutational burden/Mb had a more favorable outcome compared to tumors with a low mutational burden/Mb. Figure 1C,D shows Kaplan Meir DSS plots for *TERT* alterations and mutational burden/Mb. Supplementary Figure 3A–D shows Kaplan-Meier plots for *TERT* alterations and mutational burden/Mb for OS and MFS. Of note, the prognostic ability of mutational burden/Mb depends on all mutations

detected in tumor, not just on *TERT* alterations. Supplementary Table 5 shows details of the mutational burden/Mb in tumors with wild-type *TERT* and *TERT* alterations. Supplementary Figure 4A–C shows risk stratification based on *TERT* status and the mutational burden/Mb. Urothelial tumors with both risk factors (*TERT* alterations and low mutational burden/Mb) had the worst prognosis, while tumors with no risk factor (wild-type *TERT* and high mutational burden/Mb) had better prognosis. Tumors with one risk factor (*TERT* alterations and high mutational burden/Mb, or wild-type *TERT* and low mutational burden/Mb) had intermediate prognosis. Patient age, tumor stage, and variant histology were also predictors of OS ($p = 0.012$, <0.001, and 0.040), DSS ($p = 0.013$, <0.001, and 0.047) and MFS ($p = 0.016$, <0.001 and 0.011) in multivariate Cox regression models. CNA burden was also associated with OS ($p < 0.001$), DSS ($p < 0.001$) and MFS ($p = 0.004$) in univariate analysis, but this association was not significant after covariate adjustment in multivariate models.

In summary, the majority of *TERT* mutations are located at two promoter region hotspots. Tumors with *TERT* alterations had worse prognosis compared to tumors without *TERT* alterations, whereas tumors with a higher mutational burden/Mb had more favorable outcome compared to tumors with a low mutational burden/Mb. The results suggest that tumor genomic profiling may aid in the risk stratification of UC patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Patient summary

The majority of *TERT* gene mutations we detected in urothelial carcinoma are located at two promoter hotspots. Urothelial tumors with *TERT* alterations had worse prognosis compared to tumors without *TERT* alterations, whereas tumors with a higher mutational burden had more favorable outcome compared to tumors with low mutational burden.

Take Home Message

The majority of *TERT* mutations are located at two promoter region hotspots. Tumors with *TERT* alterations had worse prognosis compared to tumors without *TERT* alterations, whereas tumors with a higher mutational burden/Mb had a more favorable outcome compared to tumors with a low mutational burden/Mb.

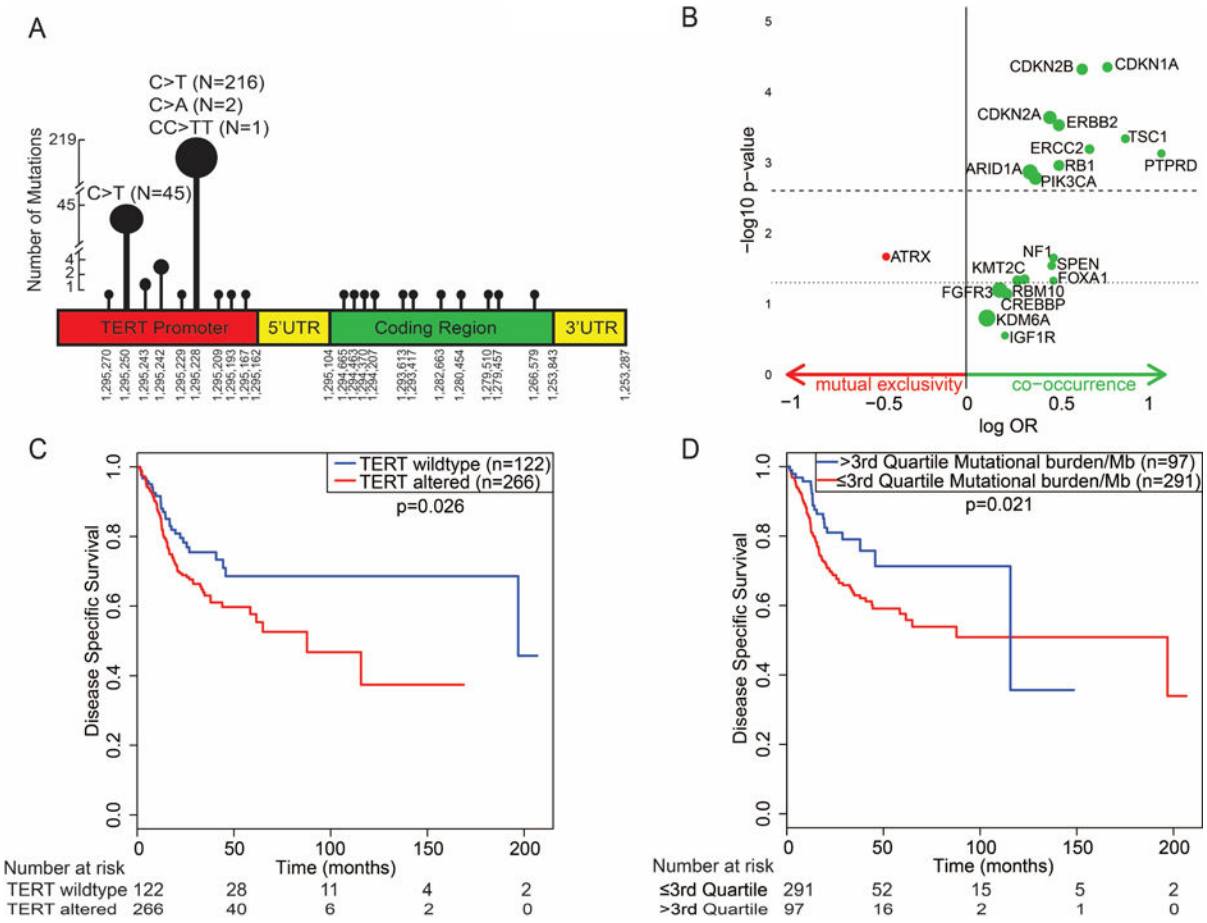

Fig. 1.

(A) Location of *TERT* mutations in the promoter region and coding regions. In our cohort of 398 urothelial tumors, 276 had a total of 286 *TERT* mutations. The most common location of TERT mutations in urothelial tumor was chromosome 5 1 295 228 (76.6%), followed by chromosome 5 1 295 250 (15.7%). Consistent with prior reports, C>T substitution was common at both of these positions. (B) Volcano plot showing co-occurrence and mutual exclusivity for *TERT* alterations and other gene alterations. The dotted line represents the unadjusted *p* value (<0.05) and the dashed line represents the adjusted *p* value (<0.0025) for multiple comparisons. The bubble size corresponds to the proportion of patients with a mutation in that gene. *ARID1A*, *PIK3CA*, *RB1*, *ERCC2*, *ERBB2*, *TSC1*, *CDKN1A*, *CDKN2A*, *CDKN2B*, and *PTPRD* alterations were significantly associated with *TERT* alteration after adjusting for multiple comparisons (all $p < 0.0025$). (C) Kaplan-Meier plots of disease-specific survival for patients with and without *TERT* alterations (promoter mutations and/or *TERT* amplification). Urothelial carcinoma with *TERT* alterations had worse disease-specific survival compared to tumors without *TERT* alteration. (D) Kaplan-Meier plots of disease-specific survival for tumor mutational burden/Mb. Urothelial carcinoma with a high mutational burden (>3rd quartile, ie, >14 mutations/Mb) had better disease-specific survival compared to tumors with a low mutational burden (≤3rd quartile, ≤14 mutations/Mb). OR = odds ratio.

Table 1

Multivariate Cox regression models for OS, DSS, and MFS

Characteristic	OS (n = 388; 120 events)		DSS (n = 388; 118 events)		MFS (n = 289; 114 events)	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
TERT alteration	2.31 (1.46–3.65)	<0.001	2.23 (1.41–3.53)	<0.001	1.63 (1.05–2.53)	0.029
Mutational burden/Mb	0.96 (0.93–0.99)	0.002	0.96 (0.93–0.99)	0.002	0.98 (0.96–1.00)	0.063
Age	1.02 (1.01–1.04)	0.012	1.02 (1.01–1.04)	0.013	1.02 (1.00–1.04)	0.016
Tumor stage						
NMIBC	Reference		Reference		Reference	
MIBC	16.77 (7.11–39.55)	<0.001	23.95 (8.93–64.23)	<0.001	11.35 (6.21–20.74)	<0.001
Localized UTUC	11.85 (4.68–30.02)	<0.001	16.03 (5.64–45.54)	<0.001	8.93 (4.79–16.66)	<0.001
Metastatic	56.09 (23.57–133.47)	<0.001	74.26 (27.57–200.02)	<0.001	–	–
Variant histology	1.54 (1.02–2.33)	0.040	1.52 (1.01–2.31)	0.047	1.77 (1.40–2.75)	0.011

OS = overall survival; DSS = disease-specific survival; MFS = metastasis-free survival; HR = hazard ratio; CI = confidence interval; NMIBC = non–muscle-invasive bladder cancer; MIBC = muscle-invasive bladder cancer;

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