DR ROHIT MEHRA (Orcid ID : 0000-0002-6955-8884)

Article type : Correspondence

Comparative Study of *TERT* Promoter Mutation Status within Spatially, Temporally and Morphologically Distinct Components of Urothelial Carcinoma

Noah A. Brown MD[a], Madelyn Lew MD[a], Helmut C. Weigelin MLS(ASCP)[a], Alon Weizer MD[b], Jeffrey Montgomery MD[b], Bryan L. Betz PhD[a] and Rohit Mehra MD[a*]

a. Department of Pathology, University of Michigan, Ann Arbor, MI, USA
b. Division of Urologic Oncology, Department of Urology, University of Michigan, Ann Arbor, MI, USA

***Correspondence:**

Rohit Mehra, MD

Department of Pathology

University of Michigan Health System

Room 2G332 UH

1500 E. Medical Center Drive

Ann Arbor, MI 48109

mrohit@med.umich.edu

Running Title: *TERT* Mutations in Urothelial Carcinoma

Keywords: bladder cancer; divergent differentiation; heterogeneity; urothelial carcinoma; TERT

Sir: Telomerase reverse transcriptase (TERT) is a ribonucleoprotein involved in maintaining the length of telomeres. In the absence of TERT expression, differentiated cells can only divide a finite number of times before undergoing cellular senescence – often referred to as the Hayflick limit. Mutations within the promoter region of *TERT* that create consensus binding sequences for ETS family transcription factors are a common mechanism by which neoplastic cells increase TERT expression and overcome this limit [1]. *TERT* promoter mutations are common in many cancer types including 60-80% of urothelial carcinomas (UC) [2,3]. Given the high frequency of these mutations in UC and absence of these mutations in non-neoplastic/benign mimics of UC [4], *TERT* promoter mutations may serve as potential biomarker for monitoring patients with a history of malignancy. Multiple studies have reported detecting *TERT* mutations in specimens commonly used for monitoring UC patients, such as urine [2,3,5]. However, in order to be a reliable marker of residual/recurrent disease, *TERT* mutation status must be a stable and uniform attribute shared among all neoplastic cells and preserved over time. To our knowledge, no previous study has compared *TERT* promoter genotypes within spatial, temporal and morphologically distinct components of UC.

In order to evaluate the stability and uniformity of *TERT* mutations within a given UC, we developed an allele-specific PCR assay targeting the most common *TERT* promoter mutations: c.-146C>T (Chr.5:1295250C>T), c.-124C>T (Chr.5:1295228C>T), c.-138_139CC>TT (Chr.5:1295242_1295243CC>TT) and c.-124_125CC>TT (Chr.5:1295228_1295229CC>TT). Using this assay, we evaluated 102 DNA samples extracted from formalin-fixed paraffin-

embedded tissues from 50 patients with invasive, high-grade UC. The age range of the patients in this cohort was 50 to 88 years, with majority of the patients demonstrating pathologic stage pT2b to pT4 at the time of cystectomy [6]. In order to determine if *TERT* mutation status varies among spatially distinct region of UC, microdissection was performed to isolate distinct regions within the same block for 19 cases and within separate blocks for 20 cases including 3 metastatic foci (Fig. 1, Supplemental Table 1). For 26 UC patients, microdissection was performed in order to separately evaluate conventional UC and components with divergent differentiation including sarcomatoid (5), nested and tubular (8), micropapillary (7), squamous (9), glandular (2), single cell/diffuse/plasmacytoid (2) and neuroendocrine (1). The variant morphologies were assigned as instructed by the WHO 2016 edition [7]. To evaluate the temporal stability of *TERT* mutations, specimens from multiple time points were evaluated for 11 patients (mean 2.9 years apart; range: 0.2 to 8.8 years). 14 single sample cases (conventional UC and divergent differentiation) were also included to establish the frequency of *TERT* promotor mutations in comparison with previous studies.

Overall, *TERT* mutations were found in in 76.0% (38/50) of UC cases – similar to previous studies [2,3]. -124C>T was the most common (34 patients), followed by -146C>T (7 patients) and a single instance of -138_-139CC>TT. *TERT* status was temporally conserved in all cases evaluated. For morphologically and spatially disparate components, we found *TERT* mutation status to be perfectly conserved in all but one case. This case harbored a -138_-139CC>TT mutation within conventional UC and a -124C>T mutation within a separate block showing squamous differentiation. These results were confirmed after re-extraction and repeated *TERT* testing. Further evaluation of these two specimens using the Ion AmpliSeq Cancer Hotspot Panel showed that both components shared a *PIK3CA* E542K mutation. However, a *PTEN* R130Q mutation was present with the squamous component but not in the conventional UC. These results suggest that while these two components are clonally related to one another, each component represents a morphologically and molecularly distinct subclonal population.

In conclusion, *TERT* promoter mutations are conserved in the majority of morphologically, spatially and temporally distinct components of a given urothelial carcinoma. These findings corroborate the notion that components of UC with divergent differentiation remain clonally related to the conventional UC. In rare cases, spatially and morphologically distinct components

of UC also show differing *TERT* genotypes. In this study, we showed that these genotypic differences reflect subclonal populations (intratumoral heterogeneity) within some cases of UC. *TERT* promoter mutations represent secondary alterations in the pathogenesis of UC and other neoplasms [1,8]. The fact that *TERT* status is spatially and temporally conserved in most cases reflects the fact that these secondary mutations generally occur early in the pathogenesis of UC [3]. Overall, *TERT* promoter mutation status is stable biomarker in most cases and may therefore be useful in disease monitoring.

Conflicts of interest

The authors declare no conflicts of interest.

Authors contribution

N. Brown, M. Lew, H. Weigelin, A. Weizer, J. Montgomery, B. Betz and R. Mehra collected the data and revised the manuscript. N. Brown, M. Lew, B. Betz and R. Mehra interpreted data and wrote the manuscript. The entire study was supervised by Noah Brown and Rohit Mehra.

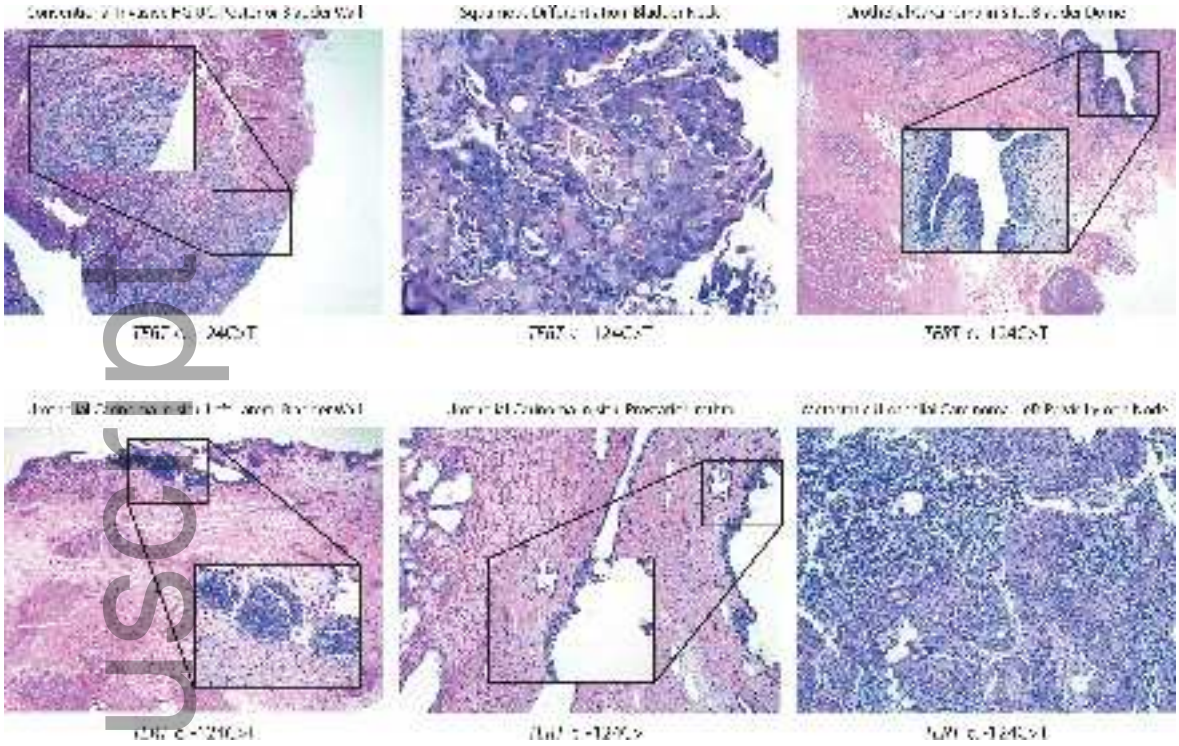
Figure Legend

FIGURE 1. An example of one patient with urothelial carcinoma (UC) in which microdissection was performed in order to isolate DNA from different components including conventional invasive high-grade UC in the posterior bladder wall, squamous differentiation in the bladder neck, urothelial carcinoma in-situ in the bladder dome, left lateral wall and prostatic urethra and squamous differentiation in a left pelvic lymph node metastasis. Identical *TERT* c.-124C>T mutations were identified in all components.

References

1. Heidenrich B, Rachakonda PS, Hemminki K, et al. *TERT* promoter mutations in cancer development. *Curr Opin Genet Dev*. 2014; **24**; 30-7.

2. Kurtis B, Zhuge J, Ojaimi C, et al. Recurrent *TERT* promoter mutations in urothelial carcinoma and potential clinical applications. *Ann Diagn Pathol.* 2016; **21**; 7-11.
3. Kinde I, Munari E, Faraj SF, et al. *TERT* promoter mutations occur early in urothelial neoplasia and are biomarkers of early disease and disease recurrence in urine. *Cancer Res*. 2013; **73**; 7162-7.
4. Zhong M, Tian W, Zhuge J, et al. Distinguishing nested variants of urothelial carcinoma from benign mimickers by *TERT* promoter mutation. *Am J Surg Pathol*. 2015; **39**; 127-31.
5. Ward DG, Baxter L, Gordon NS, et al. Multiplex PCR and next generation sequencing for the non-invasive detection of bladder cancer. *PLoS One.* 2016; **11**; e0149756.
6. Chang SS, et al. Genitourinary sites. In Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A eds. AJCC cancer staging manual, 7th edition. France: Springer; 2010; 497-505.
7. Grignon DJ, et al. Tumors of the Urinary Tract. In Moch H, Humphrey PA, Ulbright TM, Reuter VE eds. WHO Classification of Tumours of the Urinary System and Male Genital Organs. 4th ed. Lyon, France: IARC, 2016; 77-134.
8. Shain AH, Yeh I, Kovalyshyn I, et al. The genetic evolution of melanoma from precursor lesions. *N Engl J Med* 2015; **373**; 1926-36.



his_13318_f1.tiff