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Comparative study of *TERT* promoter mutation status within spatially, temporally and morphologically distinct components of urothelial carcinoma

DOI: 10.1111/his.13318

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 the absence of these mutations in non-neoplastic/benign mimics of UC,[4] *TERT* promoter mutations may serve as potential biomarkers for monitoring patients with a history of malignancy. Multiple studies have reported detecting *TERT* mutations in specimens used commonly 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 polymerase chain reaction (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 50–88 years, with the majority of the patients demonstrating pathological stage pT2b–pT4 at the time of cystectomy.[6] In order to determine if *TERT* mutation status varies among spatially distinct regions of UC, microdissection was performed to isolate distinct regions within the same block for 19 cases and within separate blocks for 20 cases, including three metastatic foci (Figure 1, Supporting information, Table S1). For 26 UC patients, microdissection was performed in order to evaluate conventional UC and components with divergent differentiation separately, including sarcomatoid (five), nested and tubular (eight), micropapillary (seven), squamous (nine), glandular (two), single cell/diffuse/plasmacytoid (two) and neuroendocrine (one). The variant morphologies were assigned as instructed by the *WHO classification of tumours of the urinary system and male genital organs*, 4th 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: 0.2–8.8 years). Fourteen 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 of 50) of UC cases, similar to previous studies;[2,3] -124C>T was the most common (34 patients), followed by -146C>T (seven patients) and a single instance of -138_-139CC>TT. *TERT* status was conserved temporally in all cases evaluated. For morphologically and spatially disparate components, we found *TERT* mutation status to be conserved perfectly in all but one case. This case harboured 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

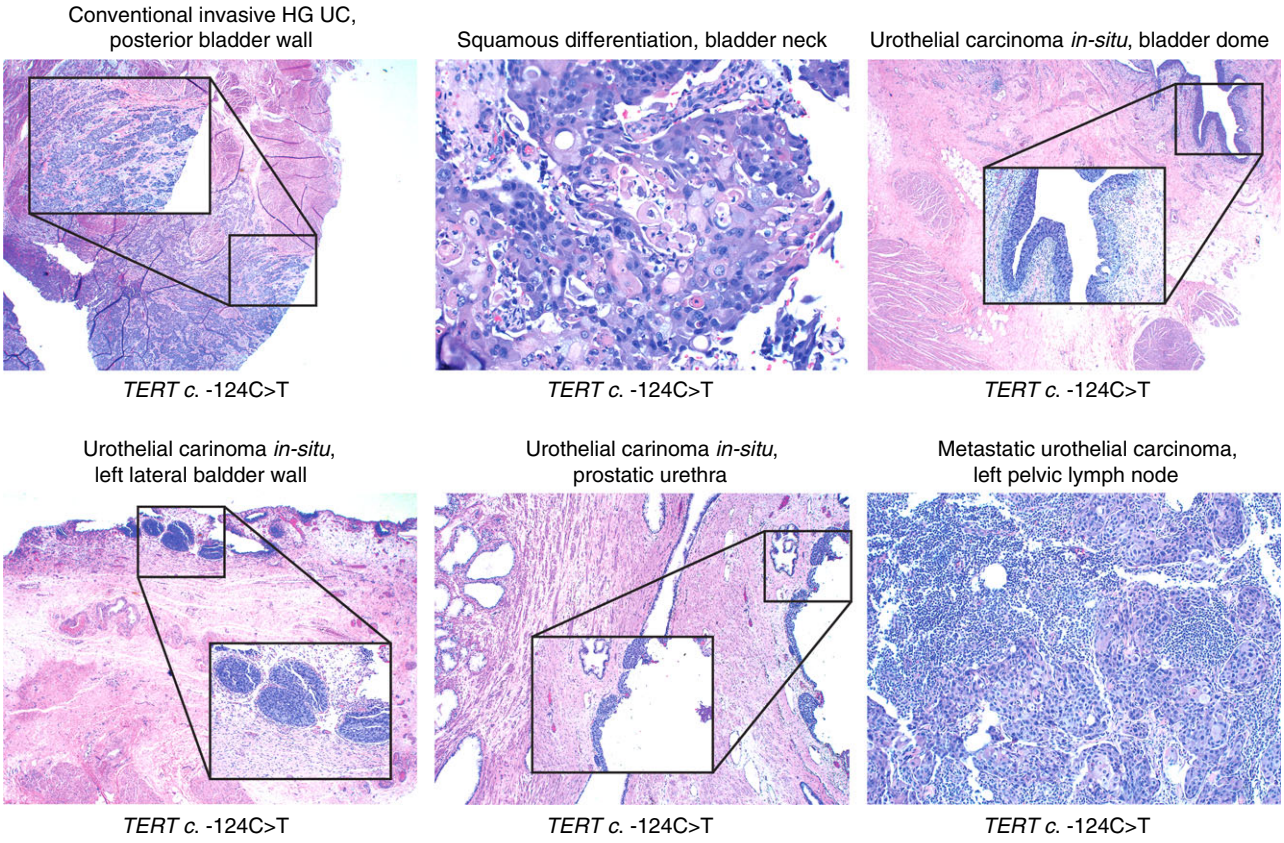

Figure 1. An example of one patient with urothelial carcinoma (UC) in which microdissection was performed in order to isolate DNA from different components, including conventional invasive high-grade UC in the posterior bladder wall, squamous differentiation in the bladder neck, urothelial carcinoma *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.

mutation was present with the squamous component, but not in the conventional UC. These results suggest that while these two components are related clonally 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 related clonally 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 genotypical 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] That *TERT* status is conserved spatially and temporally 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 a stable biomarker in most cases and may therefore be useful in disease monitoring.

Noah A Brown[1]
Madelyn Lew[1]
Helmut C Weigelin[1]
Alon Z Weizer[2]
Jeffrey S Montgomery[2]
Bryan L Betz[1]
Rohit Mehra[1]

[1]*Department of Pathology, University of Michigan, Ann Arbor, MI, USA, and* [2]*Division of Urologic Oncology, Department of Urology, University of Michigan, Ann Arbor, MI, USA*

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. *TERT* promoter mutation status in spatially, temporally and morphologically distinct components of urothelial carcinoma.

Phyllodes tumour of the urinary bladder: a report of a unique case

DOI: 10.1111/his.13344

Sir: Phyllodes tumours (PT) of the male urogenital tract are rare; to date, fewer than 100 cases have been described in the prostate, and fewer than 15 in the seminal vesicle.[1] They display histological features and clinical behaviour similar to PTs of the female breast; the lesions may be cured by surgical resection but a large proportion exhibit local recurrence, and cases of direct invasion into adjacent organs and widespread metastasis have been reported.[2] Although no single morphological feature is reliably predictive of prognosis, a combined assessment may be used for grading and prognostication, as in the breast.[2] We encountered a primary urinary bladder PT which, to our knowledge, is the first such case described in a human.

A 54-year-old man underwent partial cystectomy for a recurrent bladder tumour in September 2015 at our institution. He had presented initially with haematuria 14 years previously; since that time, he had had multiple cystoscopic resections of recurrent polypoid masses in the bladder dome. In view of the multiple episodes of recurrence, the large size of the lesion and progression on serial imaging, partial cystectomy was performed. This showed a tumour composed of broad, oedematous papillary projections, tan/grey in colour, with a firm and homogeneous cut surface.

Histological sections showed a polypoid stromoepithelial tumour arising from the mucosal compartment and arranged in broad-based papillae, many of which exhibited a club-shaped or cloverleaf-type appearance and clefting (Figure 1A). Subepithelial condensation of stromal cells was noted (Figure 1B). The stromal cells were spindled and stellate in appearance, did not show conspicuous cytological atypia, and mitotic figures were not identified (Figure 2A). Focal surface calcification and minimal superficial necrosis were present, the latter of which was related

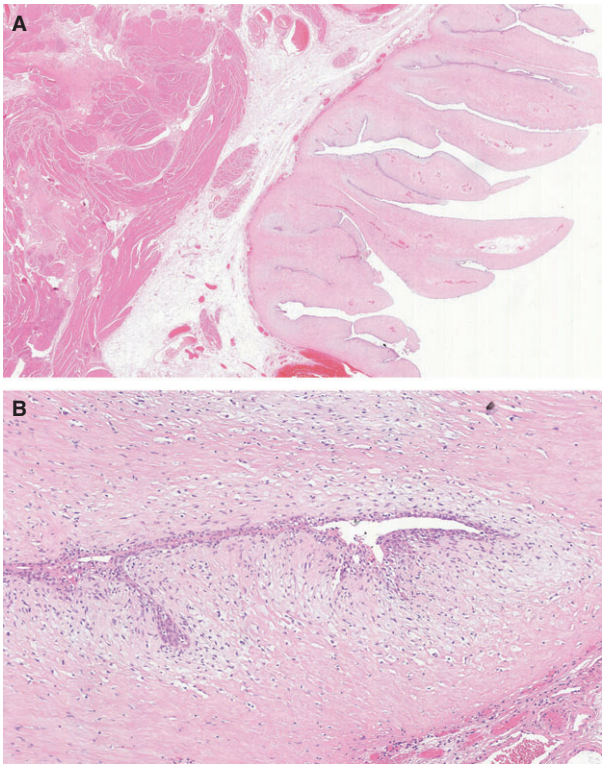

Figure 1. A, Low-power view demonstrating the polypoid, exophytic tumour projecting from the mucosal surface of the bladder. **B**, High-power view demonstrating subepithelial condensation of stromal cells.