Brief Correspondence

**TERT Promoter Mutations in Keratinizing and Nonkeratinizing Squamous Metaplasia of the Urinary Tract**

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**Abstract**

We identified urothelial tract biopsy and resection specimens with keratinizing squamous metaplasia (KSM), nonkeratinizing squamous metaplasia (NKSM), and urothelial and squamous carcinomas over a 20-yr period, focusing on cases with neurogenic lower urinary tract dysfunction (NLUTD) and/or those with spatial or temporal variation in sampling. *TERT* promoter mutations as assessed via allele-specific polymerase chain reaction were surprisingly common in our testing cohort, identified not only in 15 (94%) invasive cancer foci but also in 13 (68%) examples of KSM and seven (70%) examples of NKSM. *TERT* promoter mutations were present in 23 foci from NLUTD specimens and 11 foci from bladder diverticula, including in foci of KSM, NKSM, and unremarkable urothelium from cases with no clinical association with previous, concurrent, or subsequent cancer. Our demonstration of temporally and spatially persistent *TERT* promoter mutation in examples of KSM and NKSM in cases of bladder cancer and in morphologically benign cases with neurogenic dysfunction suggests a molecular mechanism by which such pre-neoplastic lesions can potentially progress and develop into overt carcinoma. Given the interest in *TERT* promoter mutations as a potential biomarker for the development of bladder cancer, these findings possibly explain the association between conditions with chronic urinary bladder injury (such as the natural history of NLUTD) and higher risk of bladder cancer. *TERT* promoter mutations may represent an early event in bladder cancer tumorogenesis, and our findings expand on the clinical ramifications and predictive value of *TERT* promoter mutations in this context.

**Patient summary:** Mutations in the *TERT* gene are the most common genetic changes in bladder cancer. We found that these mutations are also sometimes present in patients with chronic bladder irritation such as neurogenic bladder dysfunction and changes to the lining of the bladder that pathologists would consider “benign.” This finding might explain why such conditions are associated with the development of bladder cancer.

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**TERT** promoter mutations have been extensively studied owing to their frequency in invasive and noninvasive urinary bladder cancers as well as their potential use as biomarkers for diagnosis, prognosis, and/or recurrence. Increasingly thought of as potential “early” molecular aberrations in urothelial carcinogenesis [1,2], TERT promoter mutations have been identified in urothelial carcinomas and papillary urothelial neoplasms of low malignant potential (PUNLMP) [3–5]. In general, TERT mutations are generally thought to be absent in benign urothelium and associated reactive changes such as polypoid cystitis, von Brunn nests, cystitis cystica, cystitis glandularis, and nephrogenic adenoma, among others. During our previous work to establish the prevalence of PAX8 immunohistochemical expression and TERT promoter mutations in the nested variant of urothelial carcinoma [6], we were surprised to identify a -146C>T TERT promoter mutation in a single morphologically benign case in our control group: a cystectomy for neurogenic lower urinary tract dysfunction (NLUTD) for which histologic examination demonstrated polypoid cystitis with no morphologic atypia (at the microscopic level). To the best of our knowledge, no investigation of TERT promoter mutations in benign urothelial tissue has focused on or even explicitly included NLUTD specimens, bladder diverticula, and the keratinizing (KSM) and/or nonkeratinizing squamous metaplasia (NKSM) that can occasionally characterize epithelium in specimens from these and other clinical scenarios. Moreover, despite the long-standing notion that KSM confers a higher risk of developing subsequent carcinoma in the genitourinary tract [7], very few studies have systematically assessed this relationship using combined morphologic and molecular data [8,9], and none have provided a convincing genomic rationale/correlate for the above.

In this study, we sought to assess the presence of TERT promoter mutations in a morphologic spectrum of microdissected urothelial tissue from 35 patients with and without KSM and NKSM, including cases of NLUTD, diverticular disease, and bladder cancer.

Under institutional review board–approved protocols (with waiver of informed consent), we searched over a 20-yr time span (2000–2020) for instances of the following terms in surgical pathology reports: “keratinizing squamous metaplasia”, “nonkeratinizing squamous metaplasia”, “neurogenic bladder”, and “diverticulum”, alone and in combination with “carcinoma”, “urothelial carcinoma”, and “squamous cell carcinoma”. Morphologic diagnoses were reconfirmed by genitourinary pathologists (A.S.T. and R.M.). Squamous differentiation in the context of both squamous metaplasia and invasive carcinoma required the presence of either (1) convincing intercellular bridges or (2) definitive keratin production. The cohort of 44 surgical specimens from 35 patients (Fig. 1A) included 18 specimens from patients with urinary tract cancer (mean clinical follow-up 32 mo), 21 specimens from NLUTD patients without cancer (mean clinical follow-up 23 mo), and 5 specimens from patients with bladder diverticula without cancer (mean clinical follow-up 45 mo). Of the 18 specimens from patients with cancer, 14 were positive for malignancy; 5 cancers were associated with NLUTD and 2 were associated with diverticula. Multiple specimens (from distinct procedures) were available for 5 patients with cancer, one patient with benign NLUTD, and one patient with a benign bladder diverticulum.

We previously developed an allele-specific polymerase chain reaction 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.-125_126CC>TT (Chr. 5: 1295228_1295229CC>TT) [10]. The Supplementary material provides more information. This assay was performed on DNA samples extracted from 68 areas of microdissected, formalin-fixed paraffin-embedded tissue (specifically isolating examples of KSM, NKSM, benign urothelium, dysplastic urothelium, in situ carcinoma, and invasive carcinoma) representing 44 surgical specimens from 35 patients. Multiple morphologically distinct foci were tested when available in 15 specimens.

An organized summary of the mutation status results delineated by case type is shown in Figure 1B. We found TERT promoter mutations in 15 of 16 (94%) foci of invasive cancer, including all 7 foci of conventional urothelial carcinoma, 6 of 7 foci of extensive squamous differentiation in urothelial carcinoma, the one focus of “pure” squamous cell carcinoma, and the one focus of sarcomatoid features in urothelial carcinoma. Interestingly, our microdissected examples of KSM and NKSM also exhibited appreciable rates of mutation status positivity: 68% (13 out of 19) of KSM and 70% (7 out of 10) of NKSM foci tested. These TERT promoter mutations in KSM and NKSM existed and persisted in cases with and without associated cancer. To our surprise, in cases of NLUTD and bladder diverticu-...
associated cancer, are enriched for the presence of TERT promoter mutations. While KSM has been documented as an antecedent to clinically overt and established malignancies in the genitourinary tract (such as urothelial carcinoma and squamous cell carcinoma), our study links the presence of an established genomic insult (TERT promoter mutations) to this lesion for the first time. Interestingly, while we observed TERT promoter mutations frequently in KSM and...
NKSM, lesions that are clinically apparent to urologists and microscopically evident to pathologists, we also encountered TERT promoter mutations uncommonly in urothelium considered to be unremarkable at both the cystoscopic and microscopic/morphologic levels. This, along with the spatial and temporal preservation of TERT promoter mutations observed, suggests that these molecular aberrations may be triggered and established earlier than the associated pathologic and clinical phenotypic changes seen in such scenarios. Finally, identification of such mutations within NKSM suggests that closer clinical evaluation and follow-up of these lesions (perhaps in a fashion similar to KSM) are warranted.

Overall, this work provides insight into carcinogenesis in the settings of KSM, NKSM, and/or NLUTD. Importantly, these data suggest that the presence of a TERT promoter mutation is probably not a suitable predictive marker of overt carcinoma (urothelial or squamous) in this context, but instead represents the earlier onset of a clonal molecular process from which urothelial tumorgenesis may occur.

This shift in understanding has implications for the predictive value of TERT promoter mutation identification in surgical pathology biopsies performed on patients with urinary bladder disease. However, further studies are needed to determine the potential diagnostic and prognostic significance of these findings. Despite demonstrating spatial and temporal persistence of these mutations, we were not able to identify any cases of benign NLUTD with available biopsies preceding (by multiple years) the development of urothelial carcinoma. Similarly, we are unable to estimate the impact of TERT promoter mutations on the risk of subsequent cancer in patients with KSM and NKSM. Future work may require a sizable compilation of cases with long-term specimen availability to determine if and by how long TERT promoter mutations in biopsies may precede the development of cancer. A similar concept has been explored in urine samples, revealing a possible 10-yr lead time for urine TERT promoter mutations [1]. Studies with long-term follow-up including patients with NLUTD and known TERT promoter mutations would help to determine if noninvasive

Table 1 – TERT promoter mutations in cases with concurrent, preceding, or known subsequent carcinoma

| Pt. | Cancer location | Cancer type and TERT PM status | EDHF and TERT PM status | KSM and TERT PM status | NKSM and TERT PM status |
|-----|----------------|--------------------------------|-------------------------|------------------------|-------------------------|
| 1   | Ureter         | Invasive urothelial ca.; TERT c.-124C>T | Squamous features; TERT c.-124C>T | KSM present in concurrent and preceding specimens; both TERT WT | NKSM not identified |
| 2   | Ureter         | Invasive urothelial ca. | Squamous features; TERT c.-124C>T | KSM present in preceding sample; TERT c.-124C>T | NKSM not identified |
| 3   | Bladder wall   | Invasive urothelial ca.; TERT c.-124C>T | Squamous features; TERT c.-124C>T | KSM-A present in preceding sample; TERT c.-124C>T | NKSM not identified |
| 4   | Bladder wall   | Invasive urothelial ca.; TERT c.-124C>T | Squamous features; TERT c.-124C>T | KSM present (assay failed) | NKSM present (assay failed) |
| 5   | Bladder diverticulum | Invasive urothelial ca. | Squamous features; TERT c.-124C>T | KSM present; TERT c.-124C>T | NKSM not identified |
| 6   | Bladder wall + diverticulum | Invasive urothelial ca.; TERT c.-124C>T | Squamous features; TERT c.-124C>T | KSM present; TERT c.-124C>T | NKSM not identified |
| 7   | Bladder wall   | Invasive urothelial ca.; TERT c.-124C>T | Squamous features; TERT c.-124C>T | KSM present in adjacent bladder wall; TERT c.-124C>T | NKSM not identified |
| 8   | Bladder wall   | In situ urothelial carcinoma; TERT c.-124C>T | Sarcomatoid features; TERT c.-124C>T | KSM and KSM-A present; both areas | NKSM present; TERT c.-124C>T |
| 9   | Urethra        | SCC; TERT WT | NA | KSM present; TERT c.-124C>T | NKSM not identified |
| 10  | Bladder wall   | Invasive urothelial ca.; TERT c.-124C>T | NA | KSM not identified | NKSM not identified |
| 11  | Bladder wall   | Noninvasive urothelial ca.; TERT c.-124C>T | Squamous features; TERT c.-124C>T | KSM not identified | NKSM not identified |
| 12  | Bladder wall   | SCC; TERT c.-124C>T | NA | KSM not identified | NKSM not identified |

Pt = patient; EDHF = extensive divergent histologic features; PM = promoter mutation; ca = cancer; KSM = keratinizing squamous metaplasia; NKSM = nonkeratinizing squamous metaplasia; KSM-A = KSM with atypia; SCC = squamous cell carcinoma; WT = wild type; NA = not applicable (divergent differentiation not present).

Fig. 1 – (A) Cohort of test specimens delineated by specimen type. * Cystectomies include cystoprostatectomies and other pelvic exenterations (in cancer cases as well as partial cystectomies (in procedures for NLUTD). (B) TERT promoter mutation status in microdissected urothelium specimens isolated from all samples (n = 60 foci), NLUTD (n = 35 foci), and/or bladder diverticula (n = 11 foci). Eight foci for which our assay failed despite cleaning and repeated runs are not represented. (C) Spatial preservation of c.-124C>T TERT promoter mutation between invasive carcinoma and multiple separate foci of NKSM, KSM, KSM-A, and flat urothelial CIS. (D) Temporal perseveration of c.-124C>T TERT promoter mutation in KSM-A and multiple components of invasive carcinoma. (E) Temporal preservation of c.-124C>T TERT promoter mutation in “benign” KSM present before, at the time of, and after diverticulectomy. NLUTD = neurogenic lower urinary tract dysfunction; TUR = transurethral resection; Ca = cancer; inv. = invasive; in situ ca. = flat urothelial carcinoma in situ; NIHG = noninvasive high-grade urothelial carcinoma; CIS = flat urothelial carcinoma in situ; KSM = keratinizing squamous metaplasia; KSM-A = KSM with cytologic atypia; NKSM = nonkeratinizing squamous metaplasia; uro. = urothelium; (Con) = conventional component; (Sq) = squamous component; (Sar) = sarcomatoid component; w/ = with; w/o = without; RUO = right ureteral orifice; BX = biopsy.
molecular-based assays could serve as an effective risk assessment tool for selection of such patients for closer and/or more in-depth follow-up regimens.

**Author contributions:** Rohit Mehra had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Taylor, Chinnaiyan, Betz, Brown, Mehra.

**Acquisition of data:** Taylor, Newell, Brown, Mehra.

**Analysis and interpretation of data:** Taylor, Brown, Mehra.

**Drafting of the manuscript:** Taylor, Brown, Mehra.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euros.2021.11.007.

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