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Brief Correspondence

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

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Article info

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 urothelia from urinary bladder specimens 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.-124_125CC>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.
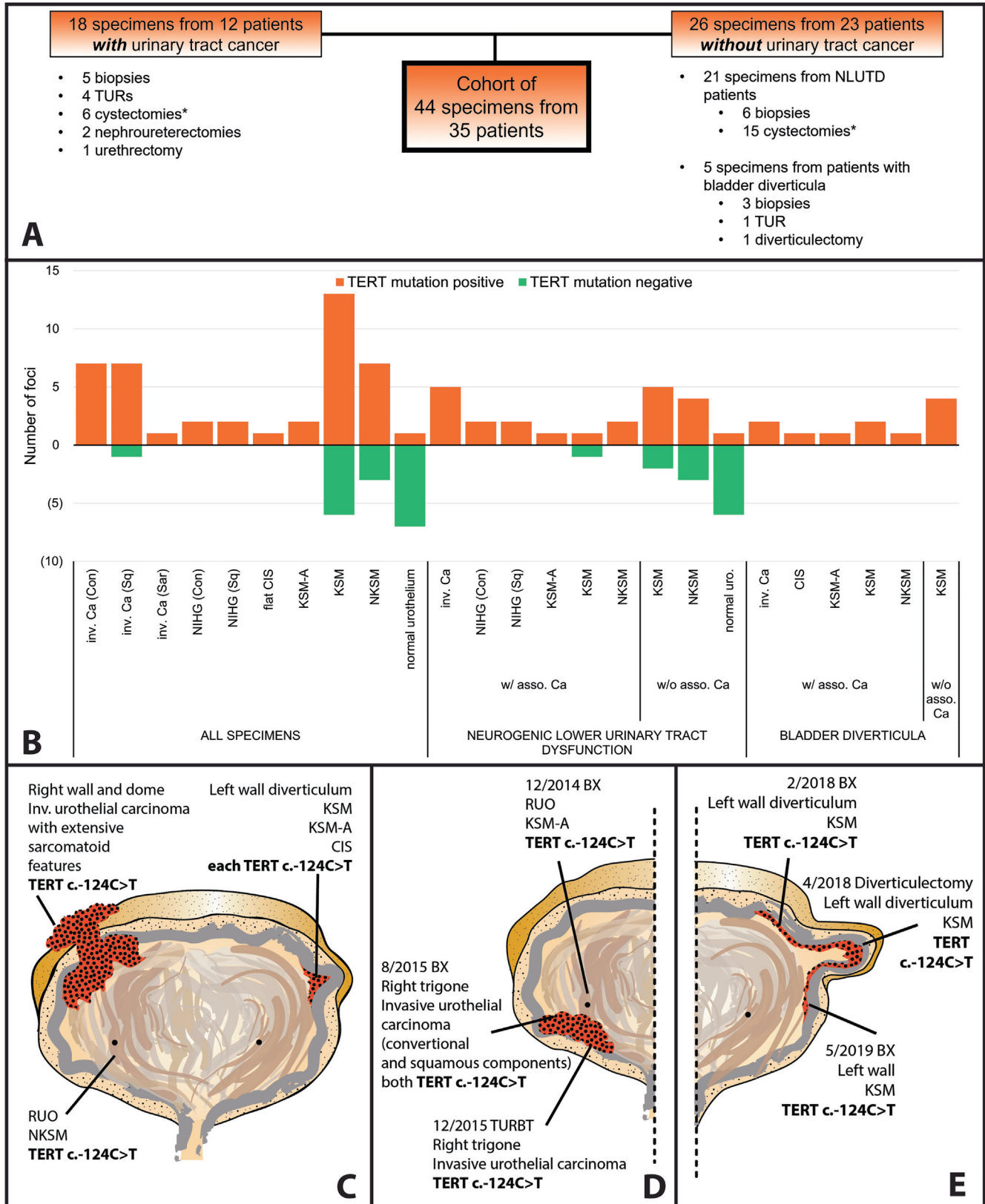
An organized summary of the mutation status results delineated by case type is shown in Figure 1B. We found *TERT* promoter mutations in 15 of 16 (94%) foci of invasive cancer, including all 7 foci of conventional urothelial carcinoma, 6 of 7 foci of extensive squamous differentiation in urothelial carcinoma, 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 diverticula with no associated preceding, concurrent, or subsequent malignancy, mutations were identified in 9 out of 11 (82%) KSM foci and 4 out of 7 (57%) NKSM foci. In addition, of 8 foci of nonmetaplastic "benign" urothelium (morphologically unremarkable at the micropscopic level), one focus from an uncomplicated NLUTD resection was found to have a *TERT* promoter mutation.

Table 1 summarizes the mutation status results for multiple morphologically distinct foci and multiple specimens (when possible) for 12 patients with associated cancer. Persistence of *TERT* promoter mutations over time was seen in 3 patients, including 2 for whom biopsies demonstrating KSM preceded (by 6–12 mo) the first histologic evidence of the patient's invasive cancer. 6 patients exhibited persistence of *TERT* promoter mutations in spatially and morphologically distinct foci, including 3 examples of KSM and 2 examples of NKSM with the same mutation as the patient's tumor. Examples of spatial and temporal preservation of mutations in multiple foci are demonstrated in Figure 1C and 1D, respectively, while temporal persistence of a positive mutation in a benign diverticulum with KSM is shown in Figure 1E. All examples of persistent mutations (from multiple foci or multiple specimens from a single patient) were concordant with respect to the specific mutation (eg, c.-124C>T), thus confirming clonal progression and tumor evolution.

Our results demonstrate that KSM and NKSM, within and outside the clinical realms of NLUTD, diverticula, and

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

Fig. A. Flowchart:

Cohort of 44 specimens from 35 patients

18 specimens from 12 patients *with* urinary tract cancer
- 5 biopsies
- 4 TURs
- 6 cystectomies*
- 2 nephroureterectomies
- 1 urethrectomy

26 specimens from 23 patients *without* urinary tract cancer
- 21 specimens from NLUTD patients
  - 6 biopsies
  - 15 cystectomies*
- 5 specimens from patients with bladder diverticula
  - 3 biopsies
  - 1 TUR
  - 1 diverticulectomy

Fig. B. Bar chart of Number of foci, TERT mutation positive (orange) and TERT mutation negative (green), across categories: ALL SPECIMENS [inv. Ca (Con), inv. Ca (Sq), inv. Ca (Sar), NIHG (Con), NIHG (Sq), flat CIS, KSM-A, KSM, NKSM, normal urothelium]; NEUROGENIC LOWER URINARY TRACT DYSFUNCTION [w/ asso. Ca: inv. Ca, NIHG (Con), NIHG (Sq), KSM-A, KSM, NKSM; w/o asso. Ca: KSM, NKSM, normal uro.]; BLADDER DIVERTICULA [w/ asso. Ca: inv. Ca, CIS, KSM-A, KSM, NKSM; w/o asso. Ca: KSM].

Fig. C.
Right wall and dome
Inv. urothelial carcinoma with extensive sarcomatoid features
TERT c.-124C>T

Left wall diverticulum
KSM
KSM-A
CIS
each TERT c.-124C>T

RUO
NKSM
TERT c.-124C>T

Fig. D.
12/2014 BX
RUO
KSM-A
TERT c.-124C>T

8/2015 BX
Right trigone
Invasive urothelial carcinoma (convertional and squamous components)
both **TERT c.-124C>T**

12/2015 TURBT
Right trigone
Invasive urothelial carcinoma
TERT c.-124C>T

Fig. E.
2/2018 BX
Left wall diverticulum
KSM
TERT c.-124C>T

4/2018 Diverticulectomy
Left wall diverticulum
KSM
TERT c.-124C>T

5/2019 BX
Left wall
KSM
TERT c.-124C>T

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	NA	KSM present (assay failed)	NKSM present (assay failed)
5	Bladder diverticulum	Invasive urothelial ca.; (assay failed)	Squamous features; *TERT* c.-124C>T	KSM present (assay failed)	NKSM not identified
6	Bladder wall + diverticulum	Invasive urothelial ca.; *TERT* c.-124C>T	NA	KSM present; *TERT* c.-124C>T	NKSM not identified
7	Bladder wall	Invasive urothelial ca.; *TERT* c.-124C>T	NA	KSM present in adjacent bladder wall; *TERT* c.-124C>T KSM also present in prostatic urethra; *TERT* WT	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 *TERT* c.-124C>T	NKSM present; *TERT* c.-124C>T
9	Urethra [a]	SCC; *TERT* WT	NA	KSM present; *TERT* WT	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 present; *TERT* c.-124C>T

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).
[a] Reactive urothelium also tested; identified to be wild type.

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 tumorogenesis 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

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.

Acknowledgements

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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