TERT promoter mutation in non-malignant urothelium of bladder is associated with recurrence in patients with non-muscle invasive bladder carcinoma.

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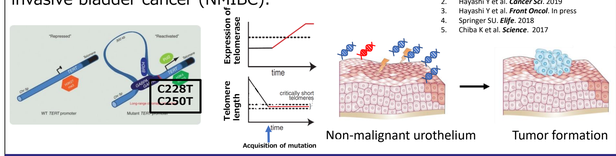
Background

TERT promoter mutations are found in 60-80% of urothelial carcinoma (UC)[1]. We previously reported that *TERT* promoter mutations are also detected in urine from patients with no evidence of cancer, and is associated with developing UC consequently[2-4]. *TERT* promoter mutations contribute to tumorigenesis in cancer cells[5]. We hypothesized that mutation in *TERT* promoter occur in non-malignant urothelium (NMU) resulting into tumor progression. In this study, we aim to investigate the status of *TERT* promoter mutation in NMU and validate its clinical utility for patients with non-muscle invasive bladder cancer (NMIBC).

1. Killela PJ, et al. ***Proc Natl Acad Sci USA***. 2013
2. Hayashi Y et al. ***Cancer Sci***. 2019
3. Hayashi Y et al. ***Front Oncol***. In press
4. Springer SU. ***Elife***. 2018
5. Chiba K et al. ***Science***. 2017



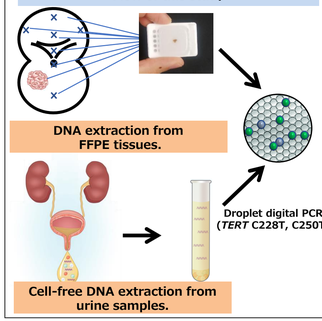
Conclusions

The *TERT* C228T mutation was detected in normal urothelium and NMU of patients with NMIBC, which may be a novel and useful prognostic factor for risk stratification of NMIBC. Analysis of systemic random biopsies for *TERT* promoter mutations in non-malignant urothelium may lead to the optimal treatment strategy for patients with NMIBC.

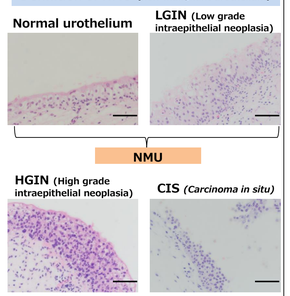
Methods

We analyzed 428 (normal urothelium:364, low grade intraepithelial neoplasia (LGIN):15, high grade intraepithelial neoplasia (HGIN):18, and biopsy proven carcinoma in situ (CIS):18) systemic bladder biopsy specimens and primary tumors from 54 NMIBC patients. NMU is defined as NU or LGIN lesions. Genome DNA extracted from formalin-fixed paraffin-embedded samples, and cell-free DNA extracted from urine of patients under surveillance after TURBT, were analyzed by droplet digital PCR on *TERT* C228T and C250T mutations. The association between the *TERT* promoter mutations and the clinical-pathological factors were analyzed using multivariate Cox regression and log-rank analysis.

Flow of this study



Definition of NMU (non-malignant urothelium)



Results

1. Characteristics of 54 patients with NMIBC.

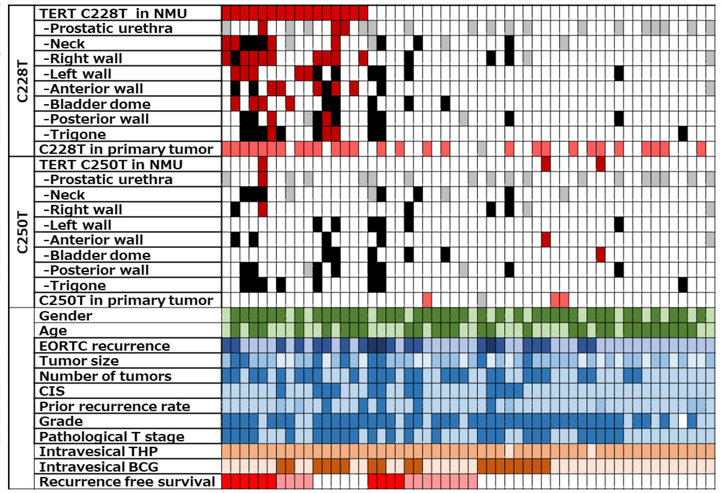
	Overall (n=54)	*TERT* C228T positive in NMU (n=16)	*TERT* C228T negative in NMU (n=38)
	n (%)	n (%)	n (%)
Gender			
Male	39 (72%)	13 (81%)	26 (68%)
Female	15 (28%)	3 (19%)	12 (32%)
Age (years)			
Median (range)	73 (33-87)	72 (59-86)	73 (33-87)
Number of tumors			
1	28 (51%)	7 (43%)	21 (55%)
2-7	25 (46%)	9 (56%)	16 (42%)
≥8	1 (1%)	0 (0%)	1 (2%)
Tumor diameter			
<3cm	46 (85%)	12 (75%)	34 (89%)
≥3cm	8 (14%)	4 (25%)	4 (10%)
Prior recurrence rate			
Primary	43 (79%)	12 (75%)	31 (81%)
≤1 recurrence/year	6 (11%)	2 (12%)	4 (10%)
>1recurrence/year	5 (9%)	2 (12%)	3 (7%)
Pathological T stage			
pTa	26 (48%)	6 (37%)	20 (52%)
pT1	25 (46%)	10 (62%)	15 (39%)
CIS	12 (22%)	4 (25%)	8 (21%)
Grade			
G1	0 (0%)	0 (0%)	0 (0%)
G2	27 (50%)	8 (50%)	19 (51%)
G3	26 (49%)	8 (50%)	18 (48%)
***TERT* C228T mutation in primary tumor**	28 (53%)	13 (81%)	15 (41%)

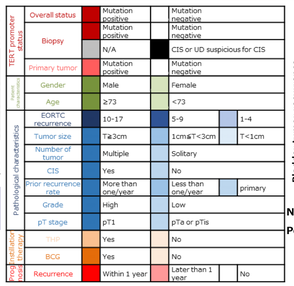
2. Frequency of *TERT* promoter mutations in biopsy specimens and primary tumors.

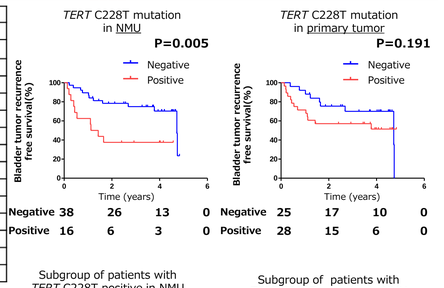
	Biopsy specimens(n=428)				Primary tumor(n=53)	
	Normal urothelium	LGIN	HGIN	CIS	pTa or pTis	pT1
***TERT* C228T mutation**	9% (31/364)	27% (4/15)	50% (9/18)	58% (18/31)	52% (15/29)	54% (13/24)
***TERT* C250T mutation**	1% (4/364)	0% (0/15)	0% (0/18)	0% (0/31)	10% (3/29)	0% (0/24)

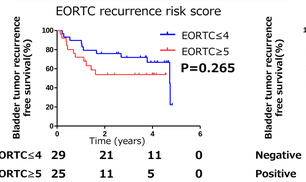
***TERT* C228T was detected 9% of normal urothelium, and 27% of LGIN.**

3. Association of clinical-pathological features and *TERT* promoter mutations.









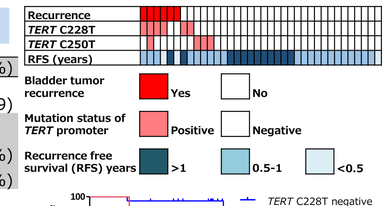
Univariate and multivariate analysis of factors associated with bladder tumor recurrence

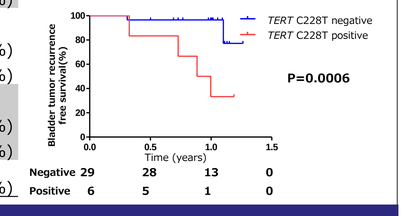
	Univariate			Multivariate model1			Multivariate model2		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
Age (≥73 vs ≤72)	0.6	0.2-1.3	0.223				0.4	0.2-1.1	0.085
pT stage (pT1 vs pTa)	1.0	0.4-2.4	0.917						
CIS (positive vs negative)	1.4	0.5-3.4	0.522						
Number of tumors (multiple vs solitary)	**3.1**	**1.3-8.2**	**0.010**	**2.8**	**1.2-7.5**	**0.020**			
Size (≥3cm vs <3cm)	2.0	0.6-5.4	0.250						
EORTC (≥5 vs ≤4)	2.1	0.9-5.0	0.142				1.9	0.7-5.6	0.227
2nd TURBT (yes vs no)	0.9	0.3-2.1	0.871						
BCG (yes vs no)	0.9	0.3-2.2	0.873				0.4	0.1-1.1	0.064
TERT C228T in NMU (positive vs negative)	**3.2**	**1.3-7.6**	**0.024**	**3.1**	**1.3-7.6**	**0.014**	**3.3**	**1.2-9.2**	**0.018**

4. Association of *TERT* promoter mutations in urinary cell-free DNA and bladder tumor recurrence.

Characteristics of 36 patients under surveillance after TURBT

	n (%)
Age, median(range)	74 (44-89)
Gender	
Male	31 (86.1%)
Female	5 (13.9%)
Cytology	
Positive	0 (0%)
Negative	36 (100%)
Mutation status	
***TERT* C228T**	6 (16.7%)
***TERT* C250T**	4 (11.1%)
Bladder tumor recurrence	6 (16.7%)





Summary of results

- ✓ *TERT* C228T mutation was detected in 9% of non-malignant urothelium, and in 30% of patients with NMIBC.
- ✓ *TERT* C228T mutation in non-malignant urothelium was significantly associated with bladder recurrence after TURBT (p=0.005).

COI disclosure

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Responsible Researcher: **Kazutoshi Fujita**

We have no financial relationships to disclose.