Supplementary Materials for

TERT promoter mutations and telomerase reactivation in urothelial cancer

Sumit Borah, Linghe Xi, Arthur J. Zaug, Natasha M. Powell, Garrett M. Dancik, Scott B. Cohen, James C. Costello, Dan Theodorescu, and Thomas R. Cech

correspondence to: thomas.cech@colorado.edu

This PDF file includes:

Materials and Methods

Growth, genotyping and CNV analysis of the UC23.

RT4, SLT4, FL3, T24 and T24T cell lines were maintained in DMEM/F12 media (Life Technologies #11320-033) supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 10% (RT4) or 5% (SLT4, FL3, T24 and T24T) fetal bovine serum (FBS). JON, 575A, SW1710, MGHU3, MGHU4, VMCUB1 and VMCUB3 cell lines were maintained in MEM media (Life Technologies #11095-080) supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 10% FBS. TCCSUP, UMUC3 and LUL2 cell lines were maintained in MEM media supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate and 10% FBS. 253J-BV, 253J-P(MDA), SCaBER, HT1197, UMUC1, UMUC6, UMUC9 and UMUC13D cell lines were maintained in MEM media supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 mM minimal non-essential amino acids, 1 mM sodium pyruvate and 10% FBS. HEK293T (ATCC #CRL-3216) and HeLa (ATCC #CCL-2) cell lines were maintained in DMEM supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 10% FBS.

Genomic DNA was harvested from each of the UC23 as per (*34*), and the *TERT* promoter was PCR amplified from each preparation for sequencing as per (*35*). CNV analysis was performed on a StepOne qPCR instrument (ABI/Life Technologies) using FAM-labeled TaqMan Assays for *TBP*, *GAPDH* and *MALAT1* and VIC/TAMARA-labeled TaqMan Assays for intron 2 or exon 16 of *TERT* (ABI/Life Technologies). The intron 2 and exon 16 probes gave similar results. For each experiment, amplification of the *TBP*, *GAPDH* or *MALAT1* gene was used for normalization by assuming these genes to be present at 2 copies/genome in all of the UC23. Analysis was performed with CopyCaller 2.0 software (ABI/Life Technologies).

Quantification of TERT mRNA and protein levels, telomerase activity and TR levels in the UC23.

TERT mRNA levels were measured by preparing cDNA using the High Capacity cDNA Reverse Transcription Kit (ABI/Life Technologies) from total RNA extracted from each of the UC23 with TRIzol reagent (Ambion). qPCR experiments were performed using iQ SybrGreen qPCR Reagent (BioRad) on a Lightcycler 480 instrument (Roche). 18S rRNA or GAPDH mRNA was used for normalization as described in (*25*). IP of TERT protein using a sheep polyclonal antibody and subsequent analysis of protein levels using a rabbit monoclonal antibody (Abcam ab32020) was performed as in (*25*). For some of the biological replicates, an IP control (IP ctrl, 0.5 μL of lysate prepared from HEK293T cells overexpressing a protein A- and FLAG-tagged, catalytically inactive mutant TERT protein) was added to each milliliter of lysate prepared from each of the UC23; the approximately equivalent levels of co-IP of this tagged protein, which electrophoresed slower than endogenous TERT on SDS-polyacrylamide gels, indicated approximately equal IP efficiencies from each of these lysates. In control experiments, addition of this small amount of overexpressed tagged protein did not compete with endogenous protein for binding by the IP antibody (data not shown). In some biological replicates, exogenously added ^{35}S-labeled TERT was used to measure IP efficiency (fig. S4). Immunoblots were visualized and quantified using a FluorChem HD2 (Alpha

Innotech). Telomerase activity in IP material was performed as in (*25*); autoradiography images were collected and analyzed using a TyphoonTrio phosphorimager scanner. One uncertainty in the quantification of TERT protein and telomerase activity is that, at least for some cell lines, activity levels were highest when the cell culture was sub-confluent and decreased as the culture approached confluence. In these cases, the amount of TERT protein still correlated very well with the level of telomerase activity, but both would be suboptimal if the cells were not harvested at the point of maximum TERT expression. TR levels in the UC23 were measured by northern blot analysis as in (*25*) using H1 RNA and/or exogenously added, *in vitro* transcribed mouse TR for normalization.

Microarray Analysis.

Microarray data for the CNUH cohort (*28*) were downloaded from the Gene Expression Omnibus (GEO; accession numbers GSE13507) (*36*) and data for the MSKCC cohort were downloaded from the supplementary material to the publication (*29*). In these cohorts, TERT mRNA from freshly frozen dissected tumors was measured by microarray. Patient characteristics for the CNUH and MSKCC cohorts are available in **Table 1** of ref. (*28*) and **Supplementary Table 1** of ref. (*29*), respectively. Informed consent was obtained from all patients (*28,29*). We restricted our analysis to the subset of patients in each cohort (CNUH, n = 35; MSKCC, n = 87) that had radical cystectomy as definitive treatment. UC23 gene expression data (*24*) were downloaded from the GEO (accession number GSE5845). Microarray probes were converted to gene symbols based on Affymetrix or Illumina annotation. For genes with multiple probes, the probe with the highest mean expression value was used.

Additional statistical analyses.

p-values were calculated using the non-parametric Wilcoxon Rank-Sum Test. Kaplan-Meier survival analysis was used to assess the prognostic value of TERT mRNA expression. HR were calculated from fitting a cox proportional hazards model and log rank *p*-values are reported.

Supplementary Text

Statistical analysis of the effect of the T24 series on correlations

The ~1.9-fold increases in both TERT protein levels and telomerase activity levels when any three of the four T24-related cell lines are omitted from the analysis are not statistically significant ($p = 0.073$ for protein levels and $p = 0.137$ for telomerase activity). However, significant differences are still observed with respect to telomere lengths upon omission of any three of these cell lines ($p = 0.02$). The identity of the cell line chosen for omission does not affect these results.

The correlation between TERT protein levels and telomerase activity remain highly significant regardless of whether only one or all four T24-related cell lines are included in the analysis; (i) $p = 0.0000041$ when all four cell lines are included, (ii) $p = 0.0015$ when only T24 is included, (iii) $p = 0.00017$ when only T24T is included, (iv) $p = 0.00053$ when only FL3 is included and (v) $p = 0.00027$ when only SLT4 is included.

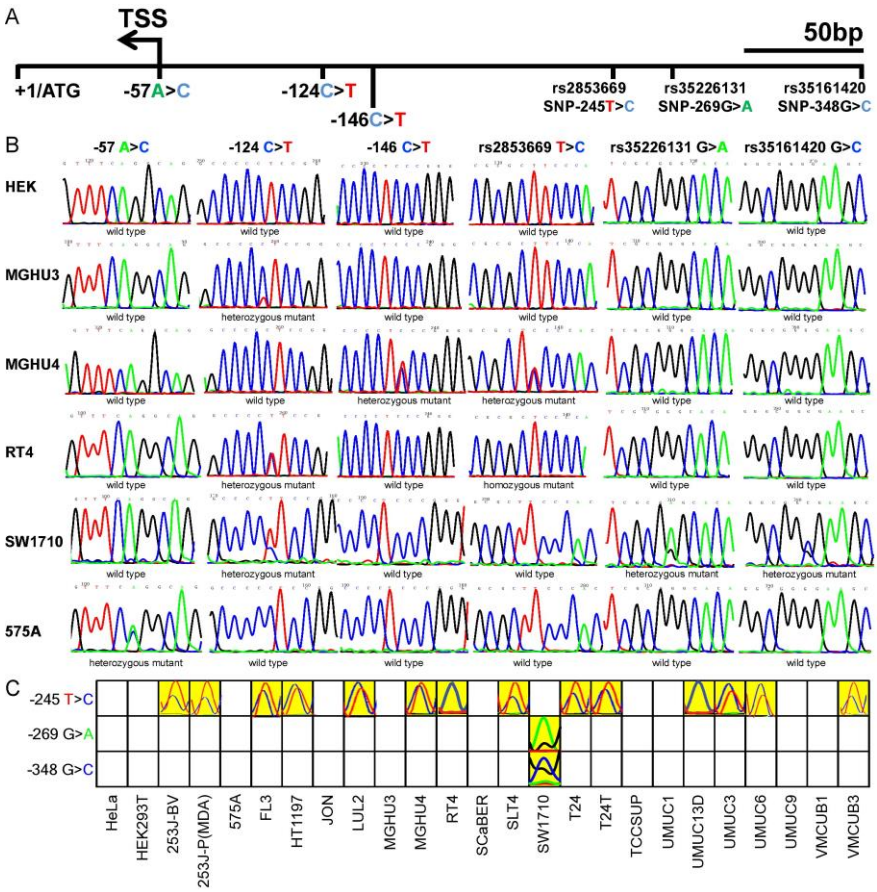

Fig. S1.

Distribution of SNPs in the proximal TERT promoters of the UC23. (**A**) Map of the proximal TERT promoter. Positions of mutations and SNPs observed in the UC23, in relation to the major annotated TSS and start codon, are indicated. (**B**) Representative chromatograms from DNA sequencing of several of the UC23. (**C**) SNPs observed in the UC23.

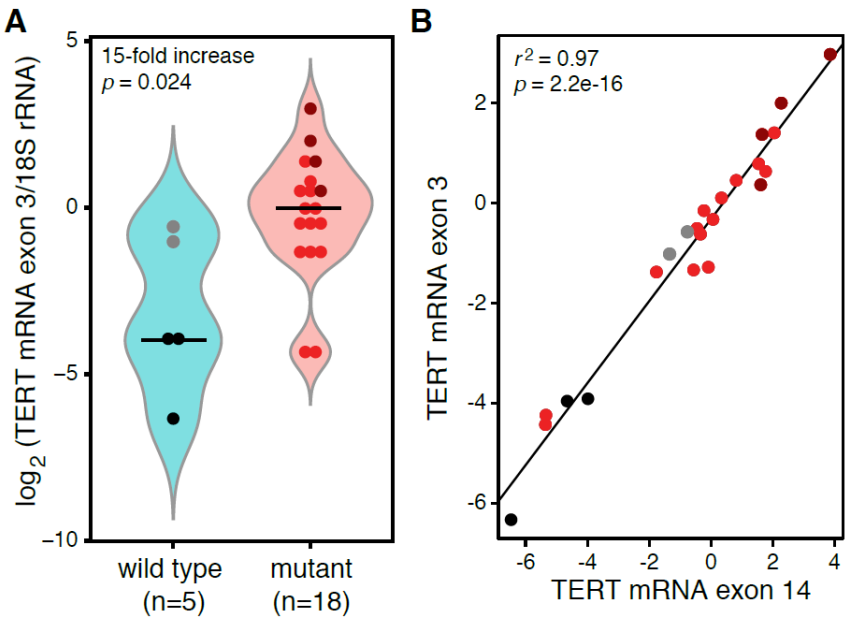

Fig. S2

TERT mRNA levels in the UC23 as a function of -124/-146 mutation status. (**A**) Amplification of exon 3 was used for detection of TERT mRNA and amplification of 18S rRNA or GAPDH mRNA was used for normalization. Horizontal bars indicate median values. (**B**) Comparison of TERT mRNA levels, as measured by qRT-PCR, using exon 3 versus exon 14 for detection. Data points are color-coded as in Fig. 2.

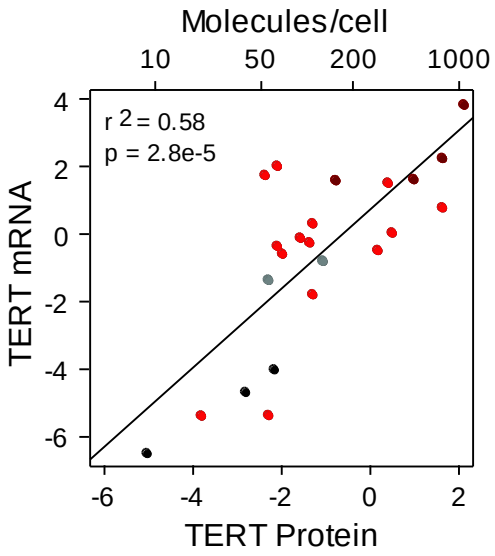

Fig. S3

Correlation between TERT mRNA and protein levels in the UC23. Data points are color-coded as in Fig. 2.

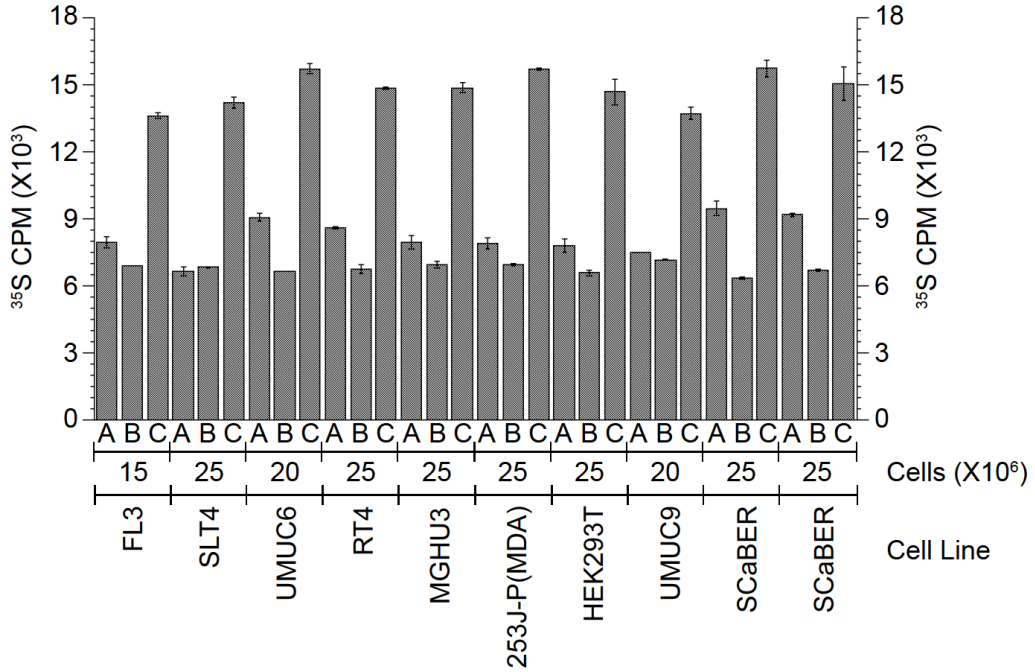

Fig. S4

TERT protein is immunopurified with roughly equal efficiency from lysates prepared from each of the UC23. TERT was *in vitro* transcribed and translated in the presence of ^{35}S-methionine and 25 µL of this reaction was added to each mL of lysate, prepared from each of the UC23, prior to incubation with the IP antibody for 30 min at 4°C. Following IP of TERT, radioactivity present in both IP and supernatant fractions was measured by liquid scintillation counting. Fraction A indicates the counts per minute (CPM) in 10 µL of extract with exogenously added ^{35}S-TERT, fraction B indicates CPM in 10 µL of depleted extract and fraction C indicates CPM in 10 µL of the final IP sample. Mean values from 3 independent experiments ± standard deviation are shown.

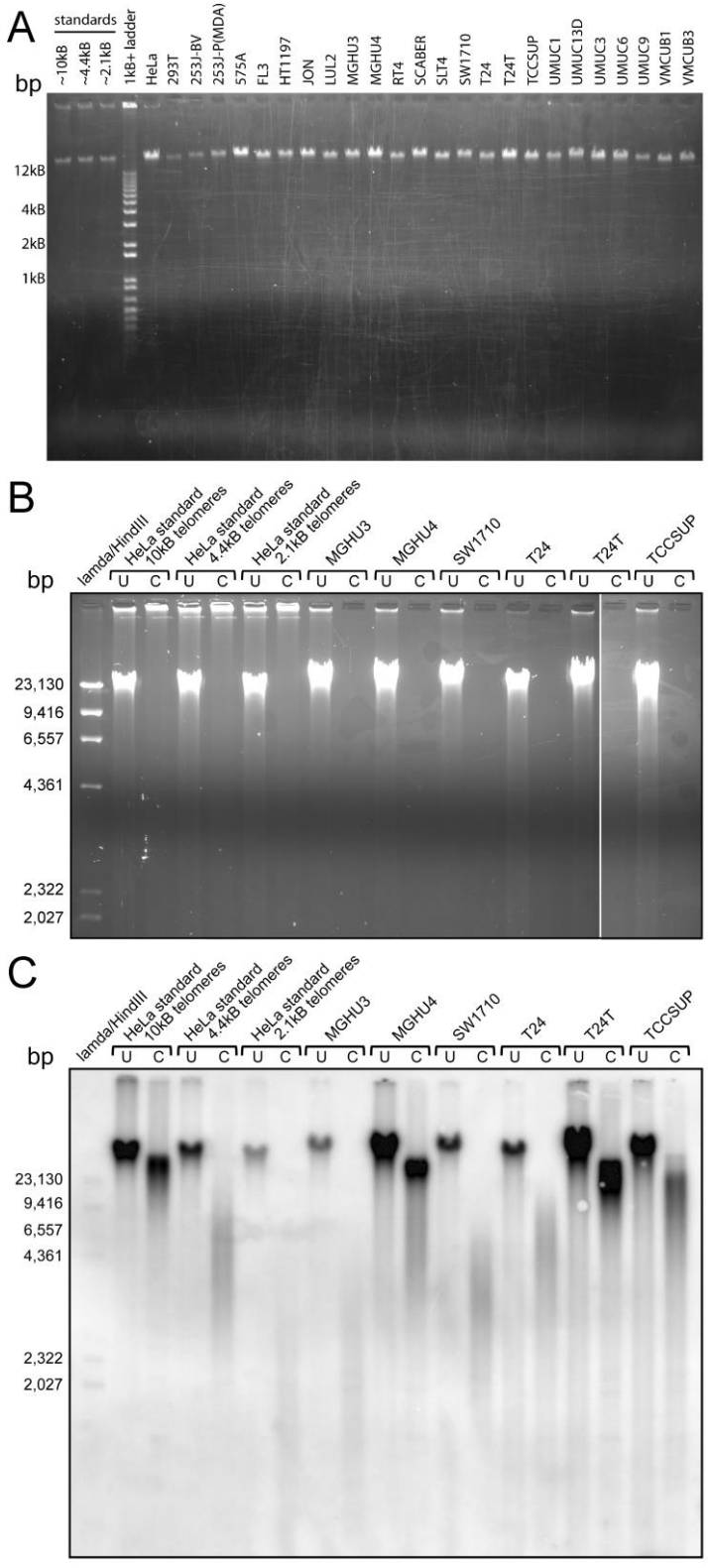
A
standards
~10kB
~4.4kB
~2.1kB
1kB+ ladder
HeLa
293T
253J-BV
253J-P(MDA)
575A
FL3
HT1197
JON
LUL2
MGHU3
MGHU4
RT4
SCABER
SLT4
SW1710
T24
T24T
TCCSUP
UMUC1
UMUC13D
UMUC3
UMUC6
UMUC9
VMCUB1
VMCUB3
bp
12kB
4kB
2kB
1kB
B
lamda/HindIII
HeLa standard 10kB telomeres
HeLa standard 4.4kB telomeres
HeLa standard 2.1kB telomeres
MGHU3
MGHU4
SW1710
T24
T24T
TCCSUP
U
C
bp
23,130
9,416
6,557
4,361
2,322
2,027
C
lamda/HindIII
HeLa standard 10kB telomeres
HeLa standard 4.4kB telomeres
HeLa standard 2.1kB telomeres
MGHU3
MGHU4
SW1710
T24
T24T
TCCSUP
U
C
bp
23,130
9,416
6,557
4,361
2,322
2,027

Fig. S5

TRF analysis of the UC23. (**A**) Integrity of total DNA extracted from each of the UC23 was assessed by electrophoresis on a 0.6% agarose/TBE gel in the presence of ethidium bromide, followed by visualization with UV light. (**B**) Completion of digestion by *Hinf*I and *Rsa*I restriction endonucleases was confirmed by visualizing electrophoresed uncut (U) and cut (C) DNA using UV light or (**C**) by capillary transfer of electrophoresed samples onto Hybond(N+) nylon membrane (GE/Amersham) and subsequent hybridization to a 5′ ^{32}P-end labeled anti-telomeric oligonucleotide probe [$(TTAGGG)_4$].

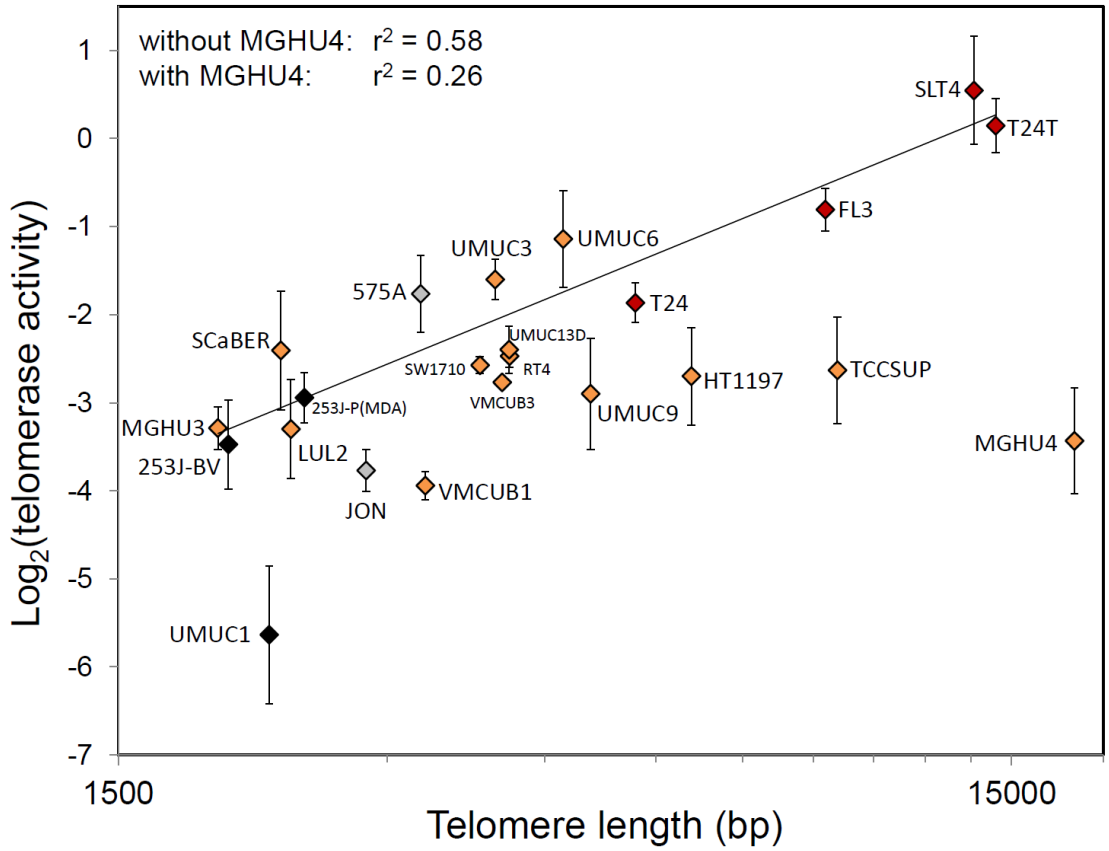

Fig. S6

Correlation between telomerase activity and telomere length in the UC23. The MGHU4 cell line was an outlier, having extremely long telomeres (Fig. 3A) despite nearly undetectable levels of TERT protein and telomerase activity. It is possible that MGHU4 cells maintain telomere length through the telomerase-independent Alternative Lengthening of Telomeres pathway, although this pathway is infrequent in UC (*32*). Data points are color-coded as in Fig. 2. Curve fit is shown without inclusion of the MGHU4 data point. Mean activity values from 2 to 9 independent experiments ± $\log_2$ error bars are shown, where $\log_2$ error = (1.443)(standard deviation/mean value).

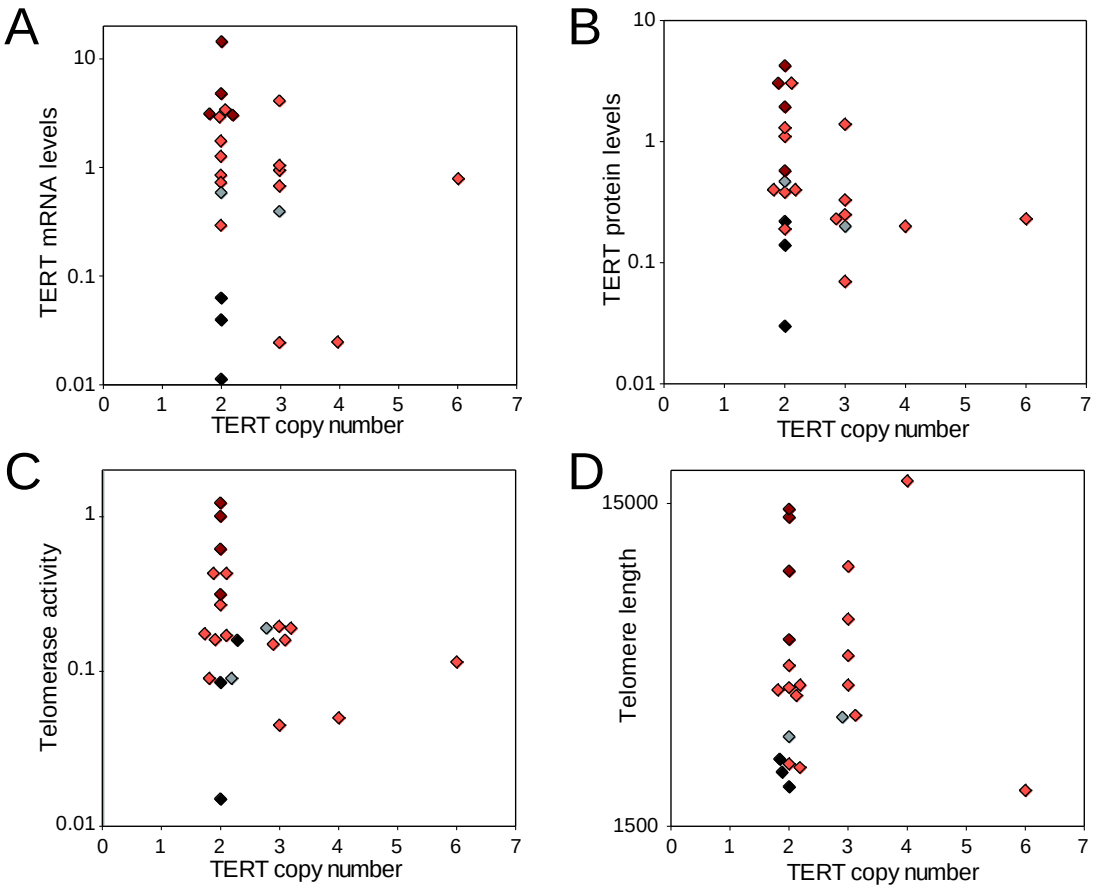

Fig. S7

TERT CNV does not correlate with (**A**) TERT mRNA levels, (**B**) TERT protein levels, (**C**) telomerase activity or (**D**) telomere length in the UC23. Data points are color-coded as in Fig. 2.

Cell Line Name	ATCC Name	Source	Lineage	Type	Morphology	Race	Tissue of origin		Stage		Grade		DSS alignment	References	Comments
							tumor origin	SRCCM alignment	tumor origin	SRCCM alignment	tumor origin	SRCCM alignment			
253J B-V	N/A	Colin Dinney	253J-P (Parent)	transitional cell carcinoma											highly tumorigenic and metastatic, a bladder line isolated after 5 serial passages in the bladder
253J-P (Parent) (MDA)	N/A	Colin Dinney	original	transitional cell carcinoma		Caucasian	BLADDER	BLADDER	T4	T2-T4	G4	G3-G4	LONG-TERM	Masters, JRW et al, *Cancer Res.*, 46:3630-3636 (1986)	poorly tumorigenic and nonmetastatic
575A	N/A	Y. Fradet/LaRue	original	transitional cell carcinoma		Caucasian	BLADDER	BLADDER		T2-T4	G3	G3-G4	LONG-TERM	Elliott, AY et al, *Cancer Res.*, 37:1279-1289 (1977)	
HTI 197	CRL-1473	ATCC	original	transitional cell carcinoma		Caucasian	BLADDER	BLADDER		Ta-T1	G3-G4	G1-G2	SHORT-TERM	Masters, JRW et al, *Cancer Res.*, 46:3630-3636 (1986) and Fanning, P et al, *Cancer Res.*, 52:1457-1462 (1992)	Tumorigenic. The cells will also grow in soft agar.
JON (JO'N)	N/A	Y. Fradet/LaRue	original	adenocarcinoma of bladder			BLADDER	BLADDER		Ta-T1		G3-G4	SHORT-TERM	Bridek, IMJ et al, *Cancer Res.*, 60:177-183 (2000)	
MGH-U3	N/A	Y. Fradet/LaRue	original	transitional cell carcinoma		Caucasian	BLADDER	BLADDER		Ta-T1	G1 (papillary)	G1-G2	SHORT-TERM	Lin CW et al, *Cancer Re s*, 45:5070-5079 (1985)	Tumorigenic, superficial like RT4
MGH-U4	N/A	Y. Fradet/LaRue	original	transitional cell carcinoma		Caucasian	BLADDER	BLADDER		Ta-T1	G1	G1-G2	SHORT-TERM	Lin CW et al, *Cancer Re s*, 45:5070-5079 (1985)	not tumorigenic
RT4	HTB-2	ATCC	original	transitional cell papilloma	epithelial	Caucasian	BLADDER	BLADDER	T2	Ta-T1	G1	G1-G2	LONG-TERM	Masters, JRW et al, *Cancer Res.*, 46:3630-3636 (1986)	The cell line is aneuploid human male, with chromosome counts in the near-diploid range. However, the near-tetraploid population may become predominant within very few passages.
SCaBER	HTB-3	ATCC	original	squamous cell carcinoma	epithelial	Black	BLADDER	BLADDER	T3	T2-T4		G3-G4	SHORT-TERM	Masters, JRW et al, *Cancer Res.*, 46:3630-3636 (1986)	2n = 46. The satellite chromosome number is hyperploid with the 2S component occurring at 0.2%
SW1710	N/A	Y. Fradet/LaRue	original	transitional cell carcinoma		Caucasian	BLADDER	BLADDER		Ta-T1	G3 (papillary)	G1-G2	LONG-TERM	Kyprios, AA et al, *Cancer Res.*, 44:3997-4005 (1984)	
T24	HTB-4	ATCC	original	transitional cell carcinoma	epithelial	Caucasian	BLADDER	BLADDER		T2-T4	G3	G3-G4	SHORT-TERM	Masters, JRW et al, *Cancer Res.*, 46:3630-3636 (1986)	hypodiploidy to hypopentaploidy; stemline 86; 2 to 4 telocentrics; 3 to 4 minutes, hypotetraploid to hypertetraploid with abnormalities
T24T	N/A	Theodorescu lab	T24		epithelial										metastatic version of T24
TCCSUP	HTB-5	ATCC	original	transitional cell carcinoma	epithelial		BLADDER	BLADDER	T4	T2-T4	G4	G3-G4	SHORT-TERM	Masters, JRW et al, *Cancer Res.*, 46:3630-3636 (1986)	(P12 and 35) hypotetraploid with marker chromosomes. The TCCSUP line was isolated in 1974 from an anaplastic transitional cell carcinoma (TCC) in the neck of the urinary bladder. The patient had a 4 month history of hematuria prior to removal of the tumor. Metastases to the bone marrow were discovered later.
UMUC1	N/A	Monica Liebert	original	lymphatic metastasis from bladder cancer	epithelial	Black	BLADDER	BLADDER		T2-T4	G2	G3-G4	SHORT-TERM	Grossman HB, et al, *J Urol* , 132:834-837 (1984)	Human transitional cell carcinoma (grade II) derived from a bladder cancer metastasis of a black male. Metastasic involvement of lymphatics. By short tandem repeat (STR)-PCR analysis the Y chromosome could not be detected in this cell line when tested at ECACC. It is a known phenomenon that due to the increased genetic instability of cancer cell lines the Y chromosome can be rearranged or lost resulting in lack of detection.
UMUC13D	N/A	Colin Dinney	original	lymphatic metastasis from bladder cancer	epithelial		BLADDER	BLADDER		Ta-T1		G1-G2	SHORT-TERM	Sabichi, A et al, *J Urol* , 175:1133-1137 (2006)	UMUC13 from Colin Dinney
UMUC3	CRL-1749	ATCC	original	transitional cell carcinoma	epithelial		BLADDER	BLADDER		T2-T4	G3-G4	G3-G4	SHORT-TERM	Grossman HB, et al, *J Urol* , 136:953-959 (1986) and Gee, JK, *Anticancer Res* , 29:3769-3775 (2009)	This is a hypertriploid human cell line. The modal chromosome number was 80, occurring in 42% of cells. Cells with 79 chromosomes also occurred at a high frequency. The rate of cells with higher ploidies was 2.5%. There were 30 or more marker chromosomes in each cell. They included der(1;W17)(q32;?), ?(1p;5p), (3q), t(7q14q), ?(2p;3p) and others. The X and N3 had single copy per cell, and others were generally two to three copies per cell. Metastasises to lung and bone.
UMUC6	N/A	Monica Liebert	original	transitional cell carcinoma	epithelial		BLADDER	BLADDER		T2-T4		G3-G4	SHORT-TERM	Grossman HB, et al, *J Urol* , 136:953-959 (1986) and Sabichi, A et al, *J Urol*, 175:1133-1137 (2006)	UM-UC-6 was obtained by transurethral resection of a bladder transitional cell carcinoma found in a male patient. UM-UC-6 was found to produce tumours in athymic nude mice.
UMUC9	N/A	Monica Liebert	original	transitional cell carcinoma	polymorphic		BLADDER	BLADDER		Ta-T1	G3	G1-G2	SHORT-TERM	Clifford , JL et al, *Cancer Epidemiol, Biomarkers, and Prev* , 10:391-395 (2001)	UM-UC-9 was derived from a transitional cel carcinoma of the bladder. UM-UC-9 was found to produce tumours in athymic nude mice.
VMCUB1	N/A	Y. Fradet/LaRue	original	transitional cell carcinoma		Caucasian	BLADDER	BLADDER		T2-T4	G2	G3-G4	SHORT-TERM	Lopez-Knowles, E, *Cancer Res*, 66:7401-7404 (2006)	
VMCUB3	N/A	Y. Fradet/LaRue	original	primary bladder tumor			BLADDER	BLADDER		Ta-T1	G3 (as xenograft)	G3-G4	LONG-TERM	Masters, J.R.W. et al, *Cancer Res* , 46:3630-3636 (1986)	Tumorigenic

Table S1.

Alternate names, source, lineage, cell type, stage and grade of tumor origin, references and additional comments for each of the UC23.

Cell Line Name	ATCC Name	Gender	Data Reference
253J-Laval	N/A	male	See 253J-P, Theodorescu Lab's DNA fingerprinting data file (compared with 253J-BV, a derivative of 253J-P), same as 253J-P
253J-P(Parent) (MDA)	N/A	male	Masters, JRW et al, *Cancer Res* , 46:3630-3636 (1986)
575A	N/A	male	Theodorescu Lab's DNA fingerprinting data file
HT1197	CRL-1473	male	Theodorescu Lab's DNA fingerprinting data file
ION	N/A	male	Theodorescu Lab's DNA fingerprinting data file
MGH-U3	N/A	male	Theodorescu Lab's DNA fingerprinting data file
MGH-U4	N/A	male	Theodorescu Lab's DNA fingerprinting data file
RT4	HTB-2	male	Theodorescu Lab's DNA fingerprinting data file
SCaBER	HTB-3	male	Theodorescu Lab's DNA fingerprinting data file
SW1710	N/A	female	Kyriazis, AA et al, *Cancer Res*., 44:3997-4005 (1984)
T24	HTB-4	female	ATCC (https://www.atcc.org/)
TCCSUP	HTB-5	female	ATCC (https://www.atcc.org/)
UMUC1	N/A	male	Health Protection Agency Culture Collections (http://hpacultures.org.uk/) citing Grossman et al, *Journal of Urology* , 132: 834-837 (1984)
UMUC13D	N/A	male	Nickerson lab's analysis of Y chromosome, Health Protection Agency Culture Collections (http://hpacultures.org.uk/) for UM-UC13: Amelogenin: X, Y
UMUC3	CRL-1749	male	ATCC (https://www.atcc.org/)
UMUC6	N/A	male	Nickerson lab's analysis of Y chromosome, Health Protection Agency Culture Collections (http://hpacultures.org.uk/): Amelogenin: X,Y
UMUC9	N/A	?	Theodorescu Lab's DNA fingerprinting data file (may be female): Amelogenin: X
VMCUB1	N/A	male	Masters, JRW et al, *Cancer Res* , 46:3630-3636 (1986)
VMCUB3	N/A	male	Masters, JRW et al, *Cancer Res* , 46:3630-3636 (1986)

Table S2.

Gender data and references for each of the UC23.

Cell Line	Gender	Data Reference	D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO	Results	
VMCUB1 p29	male	Masters, JRW et al, *Cancer Res*, 46:3630-3636 (1986)	11	10	8,11	11,12	18,19	9	X	8	11	Fingerprint match DSMZ 7/25/12	
VMCUB3 p65	male	Masters, JRW et al, *Cancer Res*, 46:3630-3636 (1986)	11	9,12	8,9	12	16	9.3	X	8	12	No fingerprint match 7/25/12	
UMUC3 p137	male	ATCC (https://www.atcc.org/)	12	8	9	8,9	17	6,9	X	10	10	Match DMSZ 3/30/12	
UMUC3-English p25	male	DNA fingerprinting data (compared with UMUC3), same as UMUC3	12	8	8,9	8,9	17	6,9	x	10	10,11	Match DMSZ 5/18/12	
SW1710 p82	female	Kyriazis, AA et al. *Cancer Res*, 44:3997-4005 (1984)	12	12	8,11	8,11	16,17	7,9,3	X	9,11	11,12	Fingerprint match DSMZ 7/25/12	
HT1197 p79	male	DNA fingerprinting data	12	11,12	11,12	12,13	16,18	6,9,3	**X,Y**	11,12	11,12	No fingerprint match 3/30/12	
TCCSUP p10	female	ATCC (https://www.atcc.org/)	12	11,14	8,9	9,11	14,16	6,9,3	X	8	10	Match DMSZ 2/28/12	
UMUC9 p113	?	DNA fingerprinting data (may be female): Ameliogenin: X	13	11	8,10	9,11	17,19	7	x	8	10	No fingerprint match 5/18/12	
575A p91	male	DNA fingerprinting data	13	13	10,12	11	14	9.3	**X,Y**	8	12	No fingerprint match 7/25/12	
UMUC1 p111	male	Health Protection Agency Culture Collections (http://hpacultures.org.uk/) citing Grossman et al, *Journal of Urology*, 132: 834-837 (1984)	13	12,14	8,11	9,11	14,15	8	X	8	12	No fingerprint match 3/30/12	matches hpa cultures database
253J-Laval p93	male	See 253J-P, DNA fingerprinting data (compared with 253J-BV, a derivative of 253J-P), same as 253J-P	10,12	9	9	9,12	17,18	6,9,3	**X**	8,11	10,12	No fingerprint match 7/25/12	
253J-BV p29	male	Masters, JRW et al, *Cancer Res*, 46:3630-3636 (1986)	10,12	9	9	9,12	17,18	6,9,3	**x**	8,11	10,12	No fingerprint match 5/18/12	
T24T p12	female	"Cousin" of T24	10,12	12	10,11	9	17	6	X	8,11	10,12	Match DMSZ 2/28/12	
T24 p8	female	ATCC (https://www.atcc.org/)	10,12	12	10,11	9	17,19	6	X	8,11	10,12	Match DMSZ 2/28/12	
RT4 Tcp3	male	DNA fingerprinting data	11,12	8	9,12	9	14,17	9,9,3	X,Y	8,11	10,12	Match DMSZ 3/30/12	
JON p32	male	DNA fingerprinting data	11,12	14	7,8	11,13	18	6,9,3	X,Y	8,9	10,11	No fingerprint match 7/25/12	
MGH-U3 p112	male	DNA fingerprinting data	11,13	12	8	9	14	7,8	X,Y	8,12	11,12	No fingerprint match 7/25/12	FGFR3 mutation confirmed
SCaBER p31	male	DNA fingerprinting data	12,13	11,12	8,10	11,12	16	7,9	X,Y	9,11	11,13	Match DMSZ 2/28/12	
MGH-U4 p88	male	DNA fingerprinting data	9,12	8,12	11	12	15	8,9	X,Y	11	12,14	No fingerprint match 7/25/12	
UMUC13D, p17	male	Nickerson lab's analysis of Y chromosome, Health Protection Agency Culture Collections (http://hpacultures.org.uk/) for UM-UC13: Amelogenin: X,Y	9,13	8,12	8,9	11	16,18	9	y	8	10,12	No fingerprint match 5/18/12	

Table S3.

Fingerprinting data and references for each of the UC23.

References

35. U. H. Frey, H. S. Bachmann, J. Peters, W. Siffert, *Nat. Protoc.* **3**, 1312-1317 (2008).
36. T. Barrett *et al.*, *Nucleic Acids Res.* **39**, D1005-D1010 (2011).