Abnormal telomerase activities are found in bladder cancer and some other urogenital tumors [1,2]. In our previous studies, we sequenced the whole genomes and transcriptomes of 97 bladder cancer patients [3]. We found upregulation of telomerase reverse transcriptase (TERT) expression and somatic mutations in the TERT promoter in these patients, though these mutations were only supported by a relatively few number of sequencing reads. To analyze the possible contributions of somatic mutations in the TERT promoter to the tumourigenesis of urogenital tumours, we sequenced the whole genomes and transcriptomes of 97 bladder cancer patients and found upregulation of TERT expression and somatic mutations in the TERT promoter in these patients, though these mutations were only supported by a relatively few number of sequencing reads. To analyze the possible contributions of somatic mutations in the TERT promoter to the tumourigenesis of urogenital tumours, we sequenced the whole genomes and transcriptomes of 97 bladder cancer patients and found upregulation of TERT expression and somatic mutations in the TERT promoter in these patients, though these mutations were only supported by a relatively few number of sequencing reads.
carcinomas, we screened, by Sanger sequencing, somatic mutations in the promoter of the TERT gene in 302 patients with urogenital cancers.

We found that 43% (130 of 302) of the urogenital tumors showed somatic mutations in the TERT promoter and the frequencies of these mutations varied greatly between different types of tumors (Table 1 and Supplemental Fig. 1). Somatic mutations in TERT promoters were identified in 55.6% and 63.7% of the bladder cancers and renal pelvis carcinomas, respectively. Low frequencies of mutations in TERT promoter were identified in the renal cell carcinomas and adrenal neoplasms (8.3% and 4.8%, respectively). In contrast, no mutation was detected in the patients with testicular cancers or prostate cancers. Two mutation hotspots at the genomic position of g.chr5:1,295,228G and g.ch5:1,295,250G>A (corresponding to −124G>A and −146G>A, respectively) were particularly common in our samples. Additionally, we also found two other low-frequency mutations in the bladder cancer patients (Table 1 and Supplemental Fig. 2).

Our findings have important implications for the diagnosis of urogenital cancers with urinary markers: The target genomic region (approximately 20 base pairs) around the mutation hotspots can be obtained easily by a single polymerase chain reaction (PCR) amplification, making it feasible to detect the somatic mutations in conventional clinical settings. However, due to the low sensitivity of Sanger sequencing, more sensitive methods (eg, PCR amplification followed by ultradep sequencing) need to be developed for the detection of somatic mutations in the exfoliated cells in urine, for the purpose of noninvasive diagnosis.

To evaluate the potential functional consequences of TERT promoter mutations, we introduced the −124G>A and −146G>A mutations into the T24 bladder cancer cell line, and then examined the functional changes caused by these mutations. The results of real-time-PCR and Western blot analyses showed that TERT expression levels were significantly unregulated in bladder cancer cells harboring the −124G>A or −146G>A mutant allele (n = 3; p < 0.01) (Fig. 1a). By performing a chromatic immunoprecipitation assay, we found that the −124G>A and −146G>A mutations strongly enhanced the combination between the TERT promoter and the transcription factor v-ets avian erythroblastsis virus E26 oncogene homolog 1 (Ets1) in the mutant T24 cells, and thereby enhanced the expression of TERT (n = 3; p < 0.01) (Fig. 1b). In wound-healing assays, we observed a significant promotion of wound closure in both −124G>A and −146G>A cells compared to the wild-type control (n = 3; p < 0.01) (Fig. 1c). This result revealed that both the −124G>A and −146G>A mutations in the TERT promoter could enhance the motility of bladder cancer cells. Our in vitro functional studies indicated that TERT promoter mutations were driver mutations that had notable influence on the expression of TERT and consequently affected the mobility of cancer cells.

We then analyzed the clinical relevance of TERT mutations by mainly focusing on bladder cancer. In our study, we found that the TERT promoter mutations were more prevalent in muscle-invasive tumors than in non–muscle-invasive tumors, and were more prevalent in bladder cancer patients with advanced tumor stages (T2–4) than those with low-stage tumors (Ta or T1). The frequency of TERT promoter mutations in patients aged ≥50 yr was obviously higher than those aged ≤50 yr (Supplemental Fig. 3a and Supplemental Table 1). Moreover, the Kaplan-Meier survival analysis revealed that the survival rate of patients with TERT mutations was significantly lower than that of patients without TERT mutations (p < 0.001) (Supplemental Fig. 3b).

These findings suggest that surveillance of urinary telomerase activities or the detection of somatic mutations would be beneficial for these high-risk populations and that more extensive retrospective and prospective studies in larger cohorts of patients are needed to evaluate the clinical application of TERT mutations.

Previous studies demonstrated that defects in other tumor-suppressing (or promoting) genes and pathways may influence the telomerase activity in human tumors [4,5]. To investigate the possible relationships between TERT and other genes or pathways that may contribute to the development of urogenital cancers, we analyzed the co-occurrence and mutual exclusivity of mutations in the TERT promoter and mutations in other genes that were previously reported to be frequently mutated in bladder cancer [3]. We observed a significant co-occurrence of TERT promoter mutations and TP53/RB1 inactive somatic mutations (odds ratio: 4.07; p = 0.001) (Fig. 1d and Supplemental Table 2).

The significant co-occurrence of TERT promoter mutations and TP53/RB1 inactive somatic mutations indicated that they may cooperatively contribute to the genesis and progression of bladder cancer. TP53 is involved in monitoring telomere and DNA integrity, and inactive mutations or deletions of TP53 gene always associate with chromosome instability [6]. The co-occurrence of TERT and TP53 gene mutations raised the possibility that TERT gene mutations may also associate with other genetic alterations that may play a role in the development and progression of bladder cancer.

### Table 1 – Prevalence of telomerase reverse transcriptase promoter mutations in urogenital tumors

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Samples analyzed, no.</th>
<th>Samples with mutations, no.</th>
<th>−124G&gt;A (%)</th>
<th>−124G&gt;T (%)</th>
<th>−146G&gt;A (%)</th>
<th>−138T−139GG&gt;AA (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer</td>
<td>216</td>
<td>92 (42.6)</td>
<td>3 (1.4)</td>
<td>22 (10.2)</td>
<td>3 (1.4)</td>
<td>120 (55.6)</td>
<td></td>
</tr>
<tr>
<td>Renal pelvic cancer</td>
<td>11</td>
<td>5 (45.5)</td>
<td>0 (0)</td>
<td>2 (18.2)</td>
<td>0 (0)</td>
<td>7 (63.7)</td>
<td></td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>24</td>
<td>2 (8.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Adrenal neoplasm</td>
<td>21</td>
<td>1 (4.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
<td></td>
</tr>
<tr>
<td>Testicular carcinoma</td>
<td>17</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>13</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>302</td>
<td>100 (33.1)</td>
<td>3 (1.0)</td>
<td>24 (7.9)</td>
<td>3 (1.0)</td>
<td>130 (43.0)</td>
<td></td>
</tr>
</tbody>
</table>
with chromosome instability. We then calculated the chromosome instability (CIN) score based on the whole-genome sequencing data from our previous studies and found that the CIN scores of tumors with TERT promoter mutations were significantly greater than those of the tumors without TERT promoter mutations ($p = 0.02$) (Fig. 1e). This result was consistent with the previous reports that TERT played an important role in the stabilization of tumor genomes [7], raising the possibility that the progenitor tumor cells harboring the TERT promoter mutations may evolve into the dominant clone by stabilizing the impaired tumor genomes.

Overall, we screened somatic mutations within the core promoter of TERT in >300 urogenital tumors and identified several high-frequency mutation hotspots that may potentially be useful for discriminating the origin of some subtypes of urogenital cancers. Nevertheless, promoter mutations only represent one of the multiple potential mechanisms leading to TERT activation and other molecular changes, such as epigenetic deregulation, still await further investigation.

**Author contributions:** Song Wu 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:** Wu.

**Acquisition of data:** P. Huang.

**Analysis and interpretation of data:** Wu, C. Li, Y. Huang.

**Drafting of the manuscript:** Lv, Y. Wang, Wu.

**Critical revision of the manuscript for important intellectual content:** X. Li.

**Statistical analysis:** Chen, W. Li.

**Obtaining funding:** Cai.

**Administrative, technical, or material support:** Tang, J. Zhou, Wu.

**Supervision:** Sun.

**Other (specify):** Lu, Gui (validation of somatic mutations); F. Zhou, D. Wang (revision and editing).
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2013.10.038.

References


