Rapid Identification of UCA1 as a Very Sensitive and Specific Unique Marker for Human Bladder Carcinoma

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Abstract

Purpose: The most common genitourinary malignancy in China is bladder transitional cell carcinoma (TCC). Early diagnosis of new and recurrent bladder cancers, followed by timely treatment, will help decrease mortality. There are currently no satisfactory markers for bladder cancer available in clinics. Better diagnostic methods are highly demanded.

Experimental Design: In this research, we have used comprehensive expressed sequence tag analysis, serial analysis of gene expression, and microarray analysis and quickly discovered a candidate marker, urothelial carcinoma associated 1 (UCA1). The UCA1 gene was characterized and its performance as a urine marker was analyzed by reverse transcription-PCR with urine sediments. A total of 212 individuals were included in this study, 94 having bladder cancers, 33 ureter/pelvic cancers, and 85 normal and other urinary tract disease controls.

Results: UCA1 was identified as a novel noncoding RNA gene dramatically up-regulated in TCC and it is the most TCC-specific gene yet identified. The full-length cDNA was 1,439 bp, and sequence analysis showed that it belonged to the human endogenous retrovirus H family. Clinical tests showed that UCA1 assay was highly specific (91.8%, 78 of 85) and very sensitive (80.9%, 76 of 94) in the diagnosis of bladder cancer and was especially valuable for superficial G2-G3 patients (sensitivity 91.1%, 41 of 45). It showed excellent differential diagnostic performance in various urinary tract diseases without TCC.

Conclusions: UCA1 is a very sensitive and specific unique marker for bladder cancer. It could have important implications in postoperative noninvasive follow-up. This research also highlights a shortcut to new cancer diagnostic assays through integration of in silico isolation methods with translational clinical tests based on RNA detection protocols.

Bladder transitional cell carcinoma (TCC) is the most common genitourinary malignancy in China (1). The major factor that accounts for the mortality of this disease is muscle invasion. Because 84% to 92% of muscle-invading lesions have their onset at the time of initial diagnosis, management approaches for these patients are unlikely to significantly reduce mortality unless they are combined with some form of early detection and reliable follow-up strategy (2). Cystoscopy, although remaining as the golden standard, is forbidding because of its invasive nature. Cytology has a low sensitivity and is subject to high variability due to interobserver reproducibility (3). The most intensively studied NMP22 and BTA tests (BTA, BTA stat, and BTA TRAK) tend to make false-positive readings in many benign genitourinary conditions (4). Therefore, finding better noninvasive methods for reliable detection of new or recurrent bladder cancer is an important topic in urology.

Global gene expression analysis has been very successful in identifying highly tumor-specific genes to serve as diagnostic targets (5–8). However, the diagnostic performance of in silico isolated genes is often poorly evaluated by translational clinical tests. Furthermore, long-term laboratory procedures for identification/cloning of protein products and production of monoclonal antibodies often retard their application in clinics. Recently, RNA assays based on reverse transcription-PCR (RT-PCR) have allowed the detection of cancer cells in the peripheral blood (9), urine sediments (10), ascites (11), bone marrow (12), and lymph nodes (13) of patients. Quite a few articles have reported that RT-PCR was a reliable method for the diagnosis of various cancer types (14–16). Nevertheless, the high sensitivity of RT-PCR necessitates that the target mRNA must be highly tumor specific to yield reliable diagnostic results. Therefore, we propose that the combination of in silico
isolation methods with clinical tests based on RT-PCR protocol might constitute a shortcut to new cancer diagnostic assays. In this research, we observed that different platforms of expression analysis often had different advantages and pitfalls, and proposed that comprehensive expression analysis of expressed sequence tags (EST), serial analysis of gene expression (SAGE), and microarray data will increase the efficacy of this procedure. We developed a specialized streamlined program for screening out TCC-specific genes based on databases of the above three platforms, and then evaluated the expression pattern of the candidate genes by RT-PCR. In this way, we identified a novel TCC-specific gene named urothelial carcinoma associated 1 (UCA1). The full-length cDNA and gene structure of UCA1 were identified and its performance as a urine marker was evaluated by prospective clinical tests through RT-PCR of voided urine sediments.

Materials and Methods

Collection of tissues. Samples of cancerous tissues, together with paired noncancerous tissues (5 cm away from tumor), were obtained during surgical resection from the first, second, and third affiliated hospitals of Peking University. Tissue samples and urine sediments were collected with written consent from patients, which were approved by the Hospital Ethic Review Committees. All samples were pathologically confirmed.

Perl programming for identification of TCC-specific genes. First, a secondary classification database for EST libraries was generated based on the Cancer Genome Anatomy Project (CGAP) information of EST libraries. The CGAP EST libraries were classified into two classes: libraries from nonfetal, nongermlinal, and nonplacental normal tissues (NT) and libraries from TCC. Second, Unigene clusters with <20 ESTs from NT libraries and >2 ESTs from TCC libraries were screened out. Third, the frequency of the best SAGE tag in NT for each candidate gene was counted based on CGAP SAGE data and Unigene clusters with <20 SAGE tags from NT were retained for further analysis. Lastly, the candidate genes were analyzed manually with Affymetrix HG-U133A/B microarray data of normal tissues downloaded from the University of California at Los Angeles public core. The secondary classification database of EST libraries and the well-annotated Perl scripts used in this article can be freely downloaded.

Reverse Transcription-PCR. Pooled cDNA from 16 normal tissues and pooled RNA of normal bladders were obtained from Clontech (Palo Alto, CA). RNAs from cancerous tissues were extracted and reverse transcribed with avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). RT-PCR was done with gene-specific primers U1 (sense, GGCACTCCITCTGTAAGACC) and L1 (antisense, AGAGAATGGATGAGCCTG; 35 cycles, 94°C, 20 seconds; 59°C, 30 seconds, 72°C, 40 seconds). A semiquantitative score of + to +++ was assigned to each RT-PCR result.

Northern blotting. Twenty micrograms total of RNA extracted from two bladder cancer tissues and a RT4 bladder papilloma cell line, together with RNA ladder, were separated using 1.5% agarose gel containing 2% formaldehyde and then blotted onto nylon membranes (Amersham Hybond-C). After stringent washes, the corresponding mRNA was detected by autoradiography for 10 and 36 hours.

Sequence analysis of UCA1. The full-length cDNA of UCA1 was assembled with CAP. BLAST was used to map the cDNA to its chromosome. BLAST was used to align the sequences. The genomic sequence of UCA1 was analyzed with a Repbase server specialized for human endogenous retrovirus (HERV) genes and aligned with a consensus genomic sequence of HERV-H (17). Open reading frame (ORF) finder and Artemis software were used to analyze the open reading frame. The coding capacity of UCA1 mRNA was tested by TESTCODE.

In vitro translation. The full-length cDNA of UCA1 with poly(A) signal was cloned into T easy vector (sp6 promoter). One-microgram of circular/linearized template was added to TNT Quick Coupled Transcription/Translation System (Promega), together with 1 32Smethionine (Amersham, Madison, WI), and incubated for 2 hours at 30°C. The control reactions with luciferase template and no template were done simultaneously. Ten microliters of translational products were separated by 20% SDS-PAGE, followed by autoradiography for 10 and 36 hours.

Cell transfection and immunofluorescence. The 5′ untranslated region and 58AA ORF of UCA1 were cloned into PCDNA3 vector with FLAG tag at the 3′ end of the ORF. The construct was transfected into 293 cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA), as well as positive control plasmid. The cells were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100 and then incubated with anti-flag M2 antibody (1:9,000, Sigma, St. Louis, MO) and FITC-conjugated antimouse immunoglobulin G.

Detection of UCA1 mRNA in urine sediments. Two hundred milliliters of first morning spontaneously voided urine were collected in sterile plastic tubes on ice and were analyzed immediately or within 2 hours of storage at 4°C. Urine samples were centrifuged at 2,000 rpm at 4°C for 15 minutes and the pelleted urothelial cells were washed twice with PBS (pH 7.6). RNA extracts of 50 to 75 μl were obtained and RT-PCR was done with the above protocol. All urine sediments included in this study were glyceraldehyde-3-phosphate dehydrogenase positive.

NMP22 and cytology. The NMP22 assay was done as described by the manufacturer (Matrient, Newton, MA). Cytology was done with smears of triplicate urine specimens stained by standard H&E staining. All slides were evaluated by two independent observers.

Statistical analysis. Using the SPSS Statistical Package, the data of clinical test were summarized in a receiver operating characteristic curve. The area under the curve and its 95% confidence interval were calculated as a measure of the discriminative efficacy of the UCA1 assay.

Results

Results of in silico screening and RT-PCR validation. A total of 55 candidate clusters were first screened out by programming

5 ftp://microarray.genetics.ucsc.edu/geneexp/public/.
6 http://download.hpa.org.
7 http://bio.igfom-firc.it/ASSEMBLY/assemble.html.
8 http://genome.ucsc.edu/cgi-bin/hgBlat.
10 http://www.girinst.org/.
12 http://www.sanger.ac.uk/Software/Artemis/.
based on EST data; after secondary analysis using SAGE and microarray data, the result was narrowed to six clusters with reconfirmed low expression in normal tissues (Supplementary data A). The six clusters were ranked according to the number of EST from TCC and RT-PCR was done to validate the TCC specificity. Between the first two genes evaluated, Hs.515223 (U133B probe: 227919_at) showed to be highly specific for TCC and we temporarily named it as UCA1.

**TCC specificity of UCA1.** The most intriguing aspect of UCA1 was its highly restricted expression in TCC when compared with both normal tissues and various other cancer types. End-point PCR of 35 cycles was far more sensitive than Northern blotting and was used to strictly evaluate the tumor specificity. Our result showed that RT-PCR failed to amplify UCA1 transcripts in the 15 pooled normal tissues other than placenta (Fig. 1A), as well as in pooled normal bladder (Fig. 1B). In contrast, UCA1 mRNA was readily detected in as high as 85.1% of bladder TCC (Fig. 1B), with about half of the cases scored "+++.

**Characterization of the UCA1 cDNA.** Northern blot analysis showed that the major transcript of UCA1 was ~1,400 bp (Fig. 2A). The 5' end of UCA1 cDNA was extended by genomic DNA sequence and a series of primers S-1 to S-5 (Supplementary data B) were designed to define the 5' end. After RT-PCR with cDNA from bladder cancerous tissue, reverse transcribed at 65°C, amplification was only achieved with S1 primer (Fig. 2B). The 3' end of UCA1 was evidenced by the alignment of multiple mRNAs and ESTs with poly(A) tails and the poly(A) signal of ATTAAA (Fig. 2C). The assembled full-length cDNA was 1,439 bp, which was consistent with the result of Northern blotting. The sequence amplified by 5'- and 3'-end primers S1 and A1 was submitted to GenBank with accession no. DQ343132.

**Chromosome mapping, gene structure, and coding capacity of UCA1.** The full-length cDNA was mapped to 19p13.12 positive strand with three exons. The cDNA sequence showed a strong similarity to the HERV-H family transcripts. The majority of the genomic sequence was also highly similar to HERV-H. The UCA1 gene was flanked by two long terminal repeats.

**Table 1.** Expression of UCA1 in various cancerous tissues and cell lines evaluated by RT-PCR.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Total</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Positive (rate, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder TCC</td>
<td>46 (1)</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>22 (1)</td>
<td>40 of 47 (85.1)</td>
</tr>
<tr>
<td>Ureter TCC</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>9 of 11 (81.8)</td>
</tr>
<tr>
<td>Pelvic TCC</td>
<td>21</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td>18 of 21 (85.7)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>10 (5)</td>
<td>4 (2)</td>
<td>3 (2)</td>
<td>2 (1)</td>
<td>1</td>
<td>9 of 15 (60)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3 of 12 (25)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>14 (2)</td>
<td>14 (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 of 16 (0)</td>
</tr>
<tr>
<td>Renal cancer</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 of 20 (0)</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>14</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2 of 14 (14.3)</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>6 (1)</td>
<td>5</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>2 of 7 (28.6)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>0 (5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 of 5 (0)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 of 7 (14.3)</td>
</tr>
<tr>
<td>Other cell lines</td>
<td>0 (3)</td>
<td>0 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 of 3 (0)</td>
</tr>
</tbody>
</table>

NOTE: Parentheses, cancer cell lines. Other cell lines are PC3 (prostate cancer), G1-102 (ovary cancer), and G1-103 (pancreatic cancer). +, ++, ++++, semiquantitative score of RT-PCR results.
repeats (LTR7A; Fig. 2D). Transcription started within the 5’ long terminal repeat and stopped 275 bp upstream of the 3’ long terminal repeat. The most striking feature of UCA1 cDNA sequence was that it was populated by an unusual number of stop codons. The TestCode value for this sequence was 0.532, which suggested that it was noncoding. The ORF nearest to the 5’ end (245-419) with adequate Kozak start context (18) translates into only 58 amino acids (6.3 kDa). We have tried to trace this protein product through Western blot analyses with polyclonal antibodies against a peptide of this pORF. However, the expected 6.3-kDa band was not found (data not shown). 

In vitro translation of UCA1 full-length cDNA also produced no protein products (Fig. 3A). Furthermore, Immunofluorescence with anti-flag antibody produced no staining in 293 cells transfected by UCA1-PCDNA3-FLAG construct (Fig. 3B).

**Diagnostic performance of UCA1 as urine marker for TCC.** UCA1 assay successfully detected 76 of 94 cases of bladder TCC (sensitivity, 80.9%; Supplementary data E). It was especially valuable in detection of superficial G2-G3 tumors; 30 of 33 superficial G2 tumors and 11 of 12 superficial G3 tumors were detected by this assay. Among the patients of bladder TCC in our study with paired results for the compared tests, UCA1 assay was more sensitive than NMP22 and cytology examinations (P < 0.05). Additionally, UCA1 assay also detected 5 of 14 ureter TCC and 9 of 19 pelvic TCC (Table 2).

In contrast, none of the 15 healthy individuals, 16 patients with benign prostatic hyperplasia, 5 patients with neurogenic bladder, or 3 patients with renal cell carcinoma showed UCA1 expression in the urine sediment. Only 5 of the 27 cases of upper urinary tract resection or reflux, 1 of 4 cystitis glandularis, and 1 of 15 lithiasis were weakly positive for UCA1 mRNA, yielding an overall specificity of 91.8% (Table 3).

The data of clinical tests were summarized by receiver operating characteristic curve to visualize the diagnostic efficacy of the UCA1 assay (Fig. 3C). The receiver operating characteristic curve showed an excellent area under the receiver operating characteristic curve of 0.882 (95% confidence interval, 0.830-0.934) and the cutoff of zero we used is very close to the optimal cutoff. The positive and negative predictive values of UCA1 assay were 91.6% and 81.3%, respectively.

**Correlation of UCA1 expression with clinicopathologic data and other urine markers.** The expression of UCA1 in 46 patients...
bladder TCC tissues was correlated with tumor stage, grade, and multicentricity. UCA1 expression was significantly correlated with multicentricity; strong expressions (+++) were seen in 58.6% of multiple tumors but only in 29.4% of solitary tumors (P < 0.05). To correlate UCA1 expression with other urine markers, 17 known urine markers were selected from the literature and a reliable probe was selected for each gene (Supplementary data F). The data matrix of the 18 markers in 18 TCC samples extracted from GSE2109 was analyzed by Spearman’s nonparametric analysis (Fig. 3D). Our result showed that UCA1 expression significantly deviated from the other urine markers (R < 0.4; P > 0.05) and is especially complementary to CFH (detected by BTA TRAK).

Discussion

Driven by the clinical demand for sensitive and reliable noninvasive methods to detect primary and recurrent bladder tumors, urologists have made decade-long efforts to discover new urine markers. However, most of the urine markers intensively studied have lower specificity than cytology and false readings are likely to be made in various genitourinary conditions (3, 4). Their expression in normal tissues is also poorly defined. Because the recent exploration of expression data has enabled the emergence of several tumor-specific genes (5, 8), we have made an effort to identify novel markers highly specific to TCC based on expression databases.

A shortcut to new cancer diagnostic methods. EST libraries currently cover more tissues than SAGE libraries pooled in the National Center for Biotechnology Information, and reconfirmation of the expression pattern with different platforms will increase the accuracy of the screening. Therefore, we used EST data for first-line screening and then validated the candidates with SAGE and microarray data. Our results showed that this comprehensive strategy makes the in silico procedure more efficient and accurate (one TCC-specific gene was identified of the first two candidates tested). The program used in this article is publicly available. We have also generated a database with in silico identified potential cancer target genes for clinical evaluation (19). This database successfully screened out 7 of 12 U.S. Food and Drug Administration–approved cancer markers with Gene ID (Supplementary data D); therefore, it might provide a good platform for rapid identification of tumor candidates.

Fig. 3. A, in vitro translation result of UCA1 full-length cDNA (autoradiography for 10 hours). No translation products were found with circular or linearized UCA1 cDNA-Teasy plasmids, which were also absent after prolonged autoradiography of 36 hours. B, 293 cells transfected by UCA1-PCDNA3-FLAG construct and positive control plasmid. Immunofluorescence with anti-flag antibody produced no staining in UCA1-PCDNA3-FLAG transfected 293 cells whereas normal staining was achieved with control plasmid. C, receiver operating characteristic curve (ROC) of UCA1 assay. D, correlation of UCA1 with other known urine markers by Spearman’s nonparametric analysis (data retrieved from GSE2109). The urine markers were sorted by R value in descending order and their expression data were normalized and visualized by red scale.

markers. Our experience also showed that, using RNA detection protocols, the potential targets might be quickly translated to clinical application after simple evaluation of their expression patterns.

Characteristics of UCA1. UCA1 is a novel gene that we have cloned and, as far as we know, it is the most TCC-specific gene reported. The stable and excellent association of UCA1 overexpression with the malignant transformation of transitional cells makes it more suitable to serve as a tumor marker than other tumor-specific genes that are likely to be affected by the genetic heterogeneity of tumors. The low expression rate in other cancer types, especially in renal cancers, makes UCA1 more discriminative in the diagnosis of TCC. Interestingly, sequence analysis shows that the UCA1 gene belongs to the HERV-H family. It contains a gag region, a protease-polymerase region, but no envelope region. UCA1 full-length cDNA is populated by an unusual number of stop codons in all of the reading frames, and our in silico and experimental results also support that this transcript is a noncoding RNA. However, as retroviruses take advantage of various unusual strategies in expression of their ORF, including translation of both spliced and unspliced RNA (20), it is possible that other spliced or unspliced forms of UCA1 transcripts might be used to produce cellular proteins.

Potential function of UCA1 in TCC. Given that the overexpression of UCA1 in TCC is pronounced and stable, dramatically specific, and significantly correlates with multifocality, it is highly unlikely that the ectopically expressed transcripts are nonfunctional RNA. These transcripts may function as mature noncoding RNAs or may be the precursor of smaller functional noncoding RNAs. Importantly, HERV genes have been shown to contribute to tumorigenesis (21–23), which also have a profound influence on the expression of adjacent genes (24, 25). Therefore, the functional significance of UCA1 in TCC deserves further elucidation. Moreover, the dramatic TCC-specific expression of UCA1 suggests a unique transcriptional regulation. As many oncogenes have been found to bind the long terminal repeat of HERVs, such as Myb (26) and CBF (27), clarification of the UCA1 long terminal repeat–interacting elements may help unravel important mechanisms for the carcinogenesis of TCC.

Diagnostic performance of UCA1. Our clinical test shows that detection of UCA1 mRNA in voided urine sediment is very sensitive and especially specific in diagnosis of bladder cancer. Seventy-six cases could be successfully detected among the 94 patients tested and only 7 of 85 patients in the control group were observed. With chronic inflammation: patients in the control group with bladder or ureteral catherization, and theoretically all patients with catheterization (AI): patients in the control group with bladder or ureteral catherization, and theoretically all patients with catheterization (AI). The results of UCA1 assay in the control group

## Table 2. Summary of diagnostic performance of UCA1 and NMP22 and cytology in TCC patients

<table>
<thead>
<tr>
<th>TCC patients (n = 127)</th>
<th>UCA1 Sensitivity (%)</th>
<th>NMP22 Sensitivity (%)</th>
<th>Cytology Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder TCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>76 of 94 (80.9)</td>
<td>34 of 65 (52.3)</td>
<td>43 of 68 (63.2)</td>
</tr>
<tr>
<td>G1 superficial</td>
<td>6 of 15 (40)</td>
<td>5 of 12 (41.7)</td>
<td>4 of 14 (28.6)</td>
</tr>
<tr>
<td>G2 superficial</td>
<td>30 of 33 (90.9)</td>
<td>4 of 15 (26.7)</td>
<td>7 of 13 (53.8)</td>
</tr>
<tr>
<td>G2 infiltrative</td>
<td>9 of 14 (64.3)</td>
<td>7 of 14 (50)</td>
<td>9 of 14 (64.3)</td>
</tr>
<tr>
<td>G3 superficial</td>
<td>11 of 12 (91.7)</td>
<td>7 of 12 (58.3)</td>
<td>9 of 12 (75)</td>
</tr>
<tr>
<td>G3 infiltrative</td>
<td>20 of 20 (100)</td>
<td>11 of 12 (91.7)</td>
<td>14 of 15 (93.3)</td>
</tr>
<tr>
<td>Ureter TCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>5 of 14 (35.7)</td>
<td>3 of 12 (25)</td>
<td>2 of 12 (16.7)</td>
</tr>
<tr>
<td>No renal atrophy</td>
<td>5 of 6 (83.3)</td>
<td>3 of 5 (60)</td>
<td>2 of 5 (40)</td>
</tr>
<tr>
<td>Pelvic TCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>9 of 19 (47.4)</td>
<td>4 of 15 (26.7)</td>
<td>3 of 15 (20)</td>
</tr>
<tr>
<td>No calicectasis</td>
<td>6 of 8 (75)</td>
<td>3 of 7 (42.9)</td>
<td>1 of 6 (16.7)</td>
</tr>
</tbody>
</table>

NOTE: Renal atrophy is the consequence of complete obstruction, which is often seen in ureter TCC, whereas calicectasis is more common in pelvic cancer as a consequence of tumor obstruction.

## Table 3. Summary of clinicopathologic data and the results of UCA1 assay in the control group

<table>
<thead>
<tr>
<th>Controls (n = 85)</th>
<th>UCA1(+)</th>
<th>Negative rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0 of 15</td>
<td>100</td>
</tr>
<tr>
<td>Upper urinary tract restriction or reflux</td>
<td>5 of 27</td>
<td>81.5</td>
</tr>
<tr>
<td>Neurogenic bladder</td>
<td>0 of 5</td>
<td>100</td>
</tr>
<tr>
<td>Cystitis glandularis</td>
<td>1 of 4</td>
<td>75</td>
</tr>
<tr>
<td>Lithisis</td>
<td>1 of 15</td>
<td>93.3</td>
</tr>
<tr>
<td>Benign prostatic hyperplasia</td>
<td>0 of 16</td>
<td>100</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>0 of 3</td>
<td>100</td>
</tr>
<tr>
<td>With chronic inflammation</td>
<td>7 of 32</td>
<td>76.1</td>
</tr>
<tr>
<td>With catheterization (AI)</td>
<td>0 of 15</td>
<td>100</td>
</tr>
<tr>
<td>Controls overall</td>
<td>7 of 85</td>
<td>91.8</td>
</tr>
</tbody>
</table>

NOTE: All of the seven positive cases were +; no ++ and +++ were observed. With chronic inflammation: patients in the control group with pathologically confirmed chronic inflammation. With catheterization (AI): patients in the control group with bladder or ureteral catheterization, and theoretically all patients with catheterization have acute inflammation (AI).
therapies (2). Therefore, the percentage of superficial G2-G3 tumors missed should be kept as low as possible. Our data show that UCA1 assay is especially valuable in the detection of this group of tumors (sensitivity, 91.1%).

Besides normal individuals, patients with various pathologic conditions of the urinary tract were selected to test the differential diagnostic performance of UCA1 assay, including upper urinary tract restriction or reflux, neurogenic bladder, cystitis glandularis, lichenoidis, benign prostatic hyperplasia, and renal cell carcinoma. UCA1 assay shows very high negative readings in all kinds of urinary tract diseases without TCC. It does not make false-positive results in the situation of inflammatory cell infiltration and bladder manipulation. Additionally, six of the seven false-positive cases have chronic inflammatory proliferative changes including one papilloma-like lesion. This indicates that UCA1 may be up-regulated in chronic proliferative conditions, which are at high risk for TCC. Therefore, UCA1 assay might help identify this critical group of patients.

The receiver operating characteristic analysis of UCA1 assay results in an area under the curve close to 0.9. This shows that UCA1 assay has a high predictive value in the diagnosis of bladder TCC. Nevertheless, a quantitative PCR assay would be necessary to determine the continuous receiver operating curve and the best cutoff. As the expression of UCA1 is unique among known urine markers and is especially complementary to the BTA TRAK test, it might favor a combination strategy for screening of bladder TCC.

**Other potential clinical applications.** Because its diagnostic performance is not influenced by inflammation, urinary tract manipulation, and other benign genitourinary conditions, UCA1 assay could have important implications during postoperative surveillance of bladder TCC. Furthermore, the ability to discriminate TCC cells from other circulating cells might enable UCA1 assay to detect circulating metastatic TCC cells. This assay may also have the potential to detect TCC from upper urinary tract. Given that 27 of 32 tissue samples of TCC from upper urinary tract overexpress UCA1, the low detection rate in urine sediments may be caused by the obstruction of upper urinary tract. In fact, five of the six ureter TCC with no obstruction could be detected by UCA1 assay. Therefore, procedures such as intrapelvic irrigation should be considered.

**Conclusion**

UCA1 is a novel noncoding RNA encoding gene highly specific to TCC. Detecting UCA1 mRNA in urine sediment is very sensitive and specific in the diagnosis of bladder TCC. It is especially valuable for detection of superficial G2-G3 tumors and shows excellent differential diagnostic performance. As the development of novel RNA detection methods such as Nucleic Acid Sequence-Based Amplification has pushed several RNA targets to clinics for the diagnosis of both pathogens (31–33) and malignancies (34), new UCA1 assays based on these recent techniques could be developed to facilitate clinical use.

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