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UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion

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Abstract **A non-protein-coding RNA, *UCA1*, has been cloned from human bladder TCC cell line BLZ-211 by using 5′ and 3′ RACE. The *UCA1* full-length cDNA was 1442 bp. RT-PCR analysis indicated that *UCA1* is an embryonic development and bladder cancer-associated RNA. The proliferative, migrative, invasive, and drug resistance behaviors of human bladder TCC cell line BLS-211 were enhanced by exogenous *UCA1* expression in vitro. Several potential target genes of *UCA1* were identified through microarray analysis. Moreover, the expression of *UCA1* also increased tumorigenic potential of BLS-211 cells in nude mice. Results from the present study suggested that *UCA1* might play a pivotal role in bladder cancer progression and embryonic development.**

Keywords: ncRNA; *UCA1*; RACE; Bladder cancer; Development; SRPK1

1. Introduction

Bladder cancer is one of the most common malignancies in China, ranking as the first frequent neoplasm of the urinary tract [1]. In most cases, bladder cancer presents as a superficial transitional cell carcinoma that is easily resectable. However, high local recurrence rates have been observed (more than 60% at 5 years and more than 80% at 15 years), and approximately 10–30% of cases will progress to invasive cancer [2,3]. In addition, approximately half of the deaths from bladder cancer result from progression. Although several potential biomarkers of disease progression and prognosis have been adopted, no single marker has emerged as the test of choice [4]. Therefore, there is a compelling need to develop reliable molecular markers to detect disease recurrence or progression to reduce the morbidity of the patients.

Recently, several studies have highlighted the role of a group of long (>400 bp) non-protein-coding RNAs (ncRNAs) in carcinogenesis and suggested that this class of genes might be used as biomarkers in cancer [5–9]. However, little is known about the involvement of ncRNAs in the progression of bladder cancer.

We previously reported a novel expressed sequence tag (EST) (Genbank accession number DR159656) isolated from two bladder transitional cell carcinoma (TCC) cell lines BLS-211 and BLZ-211 by using subtractive suppression hybridization (SSH) technique [10]. BLS-211 and BLZ-211 cells are a pair of bladder TCC cell lines which were cloned separately from the same patient's sample, but with different biological characteristics [11–13]. BLZ-211 cells have a higher invasive potential and tumorigenic property than BLS-211 cells [13]. In the present study, based on this EST, we cloned and identified a ncRNA, named *urothelial cancer associated 1* (*UCA1*, which was formerly registered as *BCIA* (bladder cancer invasion-associated gene) in GenBank nucleotide sequence databases with accession number EU334869) from BLZ-211 cells, and analyzed the tissue expression pattern and the roles of UCA1 in order to explore the molecular basis responsible for a functional role in bladder tumor progression and embryonic development.

2. Materials and methods

2.1. Cell culture, tissues collection and RNA extraction

Human bladder TCC cell lines BLS-211 and BLZ-211 were cultured in RPMI 1640 medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% bovine calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2. All tissue samples were obtained during surgical operation from the First Affiliated Hospital, School of Medicine of Xi'an Jiaotong University, then immediately snap-frozen in liquid nitrogen and stored at −80 °C. All samples were pathologically confirmed and collected with written consent from each patient. It was approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine of Xi'an Jiaotong University. Total RNA from cells and tissues were extracted using TRIzol reagent (Invitrogen, USA). RNA concentration and integrity were determined by spectrophotometry and standard RNA gel electrophoresis.

2.2. Northern blotting

Total RNA (20 μg) of BLS-211 and BLZ-211 cells, together with RNA ladder, were size separated by electrophoresis on 1% denaturing formaldehyde agarose-MOPS gel and then blotted onto nylon membranes (Hybond N, Amersham). The membranes were hybridized to the [α-^{32}P] dCTP-labeled probe (DR159656) overnight at 42 °C. Filters

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Abbreviations: 18S, 18S rRNA; AURKC, a urora kinase C; BCIA, bladder cancer invasion-associated gene; bp, base pair; CYP1A1, cytochrome P450, 1A1; DMSO, dimethyl sulfoxide; EST, expressed sequence tag; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HERV, human endogenous retrovirus; HULC, highly up-regulated in liver cancer; MBD3, methyl-CpG binding domain protein 3; LTR, long terminal repeat; MLT1A, mammalian LTR transposon1A; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; ncRNA, non-coding RNA; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription polymerase chain reaction; SRPK1, SR (serine/arginine-rich) protein-specific kinase 1; SSH, suppression subtractive hybridization; TCC, transitional cell carcinoma; UCA1, urothelial cancer associated 1; WNT6, wingless-type MMTV integration site family, member 6

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were washed in 1 × SSC, 0.1% SDS for 20 min at 68 °C, and three times in 0.2 × SSC, 0.1% SDS for 20 min at 68 °C. Hybridized RNA signals were detected by autoradiography. The 18S rRNA (18S) was also detected as control by hybridization with a 252 bp cDNA probe (M10098, 1565–1816).

2.3. Rapid amplification of cDNA ends (5′ and 3′ RACE)

One micrograms of total RNA of BLZ-211 cells was purified further by treating with RNase-Free DNase I (Takara, Dalian, China), then reverse transcribed with the SMART RACE cDNA Amplification Kit (Clontech) according to manufacturer's instructions. Specific 5′ and 3′ RACE cDNA ends were amplified with the universal primer mix provided by kit and gene specific primers (GSPs) with the advantage 2 PCR polymerase mix (Clontech). The PCR products were subcloned into pGEM-T Easy vector (Promega) and several recombinant clones were isolated for sequencing. The GSP sequences are 5′-RACE-GSP1: 5′-GTCCAGAGGAACGGATGAAGCCTGC-3′; 3′-RACE-GSP2: 5′-CTACAGCCTCAATGGACCAGACCCTACC-3′.

2.4. Sequence analysis of UCA1

The full-length cDNA of *UCA1* was assembled with DNAMAN version 6. BLAT was used to map the cDNA to chromosome. BLAST was used to align the sequences. Open reading frame (ORF) finder software was used to analyze ORF. The coding capacity of *UCA1* was tested by TESTCODE.

2.5. Expression analysis of UCA1 RNA in various tissues by RT-PCR

One micrograms of total RNA of every tissue sample was reverse transcribed by using ImProm II reverse transcriptase (Promega). Primer sequences for 30 cycles of PCR amplification were as follows: *UCA1* (forward: 5′-CTCTCCATTGGGTTCACCATTC-3′, reverse: 5′-GCGGCAGGTCTTAAGAGATGAG-3′), 18S (forward: 5′-CAGCCACCCGAGATTGAGCA-3′, reverse: 5′-TAGTAGCGACGGGCGGTGTG-3′). Annealing temperature was at 59 °C.

2.6. Stable transfection of UCA1 cDNA

The full-length cDNA of *UCA1* was amplified using primers (forward: 5′-CGGGATCCTGACATTCTTCTGGACAATGAG-3′, reverse: 5′-CCGGAATTCGCATATTAGCTTTAATGTAGGTGGC-3′). The PCR product was purified and digested with BamHI and EcoRI restriction enzymes, subcloned into the pcDNA3.1 mammalian expression vector (Invitrogen) and sequenced. Thereafter, the pcDNA/*UCA1* construct was transfected into BLS-211 cells with lipofectamine2000 for 24 h and selected with 150 μg/ml G418 for 3 weeks. Transfection with pcDNA3.1 empty vector (MOCK) acted as a control. The positive clone was identified by reverse transcription polymerase chain reaction (RT-PCR) for *UCA1* and neo gene expression. The primers of neo are as follows: forward, 5′-ACAAGATGGATTGCACGCAGG-3′; reverse, 5′-TTCTCGGCAGGAGCAAGGTGA-3′. Annealing temperature was at 58 °C.

2.7. Cell proliferation, colony formation, and ex vivo tumorigenic assay

Cell proliferation was assessed by using MTT (Amresco, Solon, OH, USA) assays. Briefly, 4×10^3 cells were separately seeded at the same time into 96-well culture plates and then routinely cultured for 7 days. Twenty microliters of (5 mg/ml) 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well. The cells were cultured at 37 °C for 4 h, 150 μl dimethyl sulfoxide (DMSO) was added, and the 490 nm wave-length absorption value was measured. All experiments were performed in triplicate and repeated three times.

Colony formation was assessed as follows: cells were trypsinized into a single cell suspension. A total of 100 cells were plated in each well of 6-well plates and kept for 14 days in RPMI 1640 supplemented with 10% bovine calf serum containing 150 μg/ml G418 to allow colony formation. Cell clones over 50 cells were counted using a grid. Three independent experiments were performed.

For in vivo tumorigenicity, pcDNA/*UCA1* and pcDNA3.1 stable transfected BLS-211 Cells were trypsinized, counted, and centrifuged and re-suspended into sterile PBS (1×), 200 μl cells (8×10^6 cells) of the suspension was injected into left posterior limb subcutaneous region of athymic nude mice (4–6 weeks of age). On the seventh day post injection, tumors began to develop and their volumes were measured routinely using a caliper. Four weeks post injection, all mice were killed, tumor sizes, weights and the expression of *UCA1* RNA were detected. The permission for the mouse experiments was obtained from the Institutional Animal Care and Use committee of Xi'an Jiaotong University and conducted in accordance with the European community council directive 68/609/EEC guidelines.

2.8. Cell invasion, motility and drug resistance assay

Invasion assay was done in a 24-well Millicell chamber. The 8 μm pore inserts were coated with 15 μg of Matrigel (Becton Dickinson Labware, Bedford, MA). 5×10^5 cells were added to coated filters in 100 μl of serum-free medium in triplicate wells. Six hundred microliters of RPMI 1640 media containing 20% fetal bovine serum was added to the lower chamber as chemoattractant. After 24 h at 37 °C in a 5% CO_2 incubator, the Matrigel coating on the upper surface of the filter was wiped off using a cotton swab. Cells that migrated through the filters were fixed in 2.5% glutaraldehyde for 30 min, stained with Giemsa, photographed, and counted. The motility assay was conducted in a similar fashion without coating with Matrigel. The plates were incubated for 8 h then detected. Each experiment was carried out in triplicate.

Cisplatin resistance was detected as follows: 10000 pcDNA/*UCA1* and pcDNA3.1 stable transfection cells per well were seeded separately in a 96-well plate for 24 h, then exposed to various concentrations of drugs (0, 5, 10, 20, 40, 80 μM) for 48 h, and detected by MTT assay. All experiments were performed in quadruplicate and repeated two times.

2.9. Microarray mRNA expression analysis and verification of selected genes by real-time PCR analysis

Total RNAs were isolated from BLS-211 cells that stable transfected by pcDNA/*UCA1* or pcDNA3.1 plasmids, and were subjected to reverse transcription, labeling and hybridization to Agilent Human 1A Microarray (V2) G4110B gene chip arrays (Agilent Technologies, Palo Alto, CA), containing about 22000 transcripts and variants, including 18700 well characterized human genes. Genes whose expression changed by at least twofold (pcDNA/*UCA1* versus pcDNA3.1) are selected. Then we identified several different up- and down-regulated genes (Table 1) with Quantitative real-time PCR on an ABI PRISMR 7300 Se-

Table 1
Gene name and primer information for real-time PCR

Gene name	Primer sequence	Position (mRNA)	Product (bp)
AURKC (NM_001015878)	5′-CCCAATATCCTGCGCCTGTATAAC-3′	490–513	166
	5′-AGGTCAGGGCATCTGCCAAC-3′	636–655	
CYP1A1 (NM_000499)	5′-GCCAAGAGTGAAGGGAAGAGA-3	2094–2114	134
	5′-AGGAAGGGCAGAGGAATGTG-3′	2208–2227	
WNT6 (NM_006522)	5′-CTGGAATTGCTCCAGCCACA-3′	467–487	87
	5′-GCAGTGATGGCGAACACGA-3′	535–553	
SRPK1 (NM_003137)	5′-AGCAAGCCACCAGTGAGGCTA-3′	3586–3606	159
	5′-GAATGGCTGGCCAGTTACTCAGA-3′	3722–3744	
MBD3 (NM_003926)	5′-GGCAAGATGCTGATGAGCAAGA-3′	221–242	139
	5′-TCACCGGCTGCTTGAAGATG-3′	340–359	
GAPDH (NM_004360)	5′-AGGTCGGAGTCAACGGATTTG-3′	66–86	551
	5′-GTGATGGCATGGACTGTGGT-3	597–616	

quence Detection System (Applied Biosystems, Foster City, CA, USA) using SYBR® Premix Ex Taq™ Kit (Takara Biotechnology Co. Ltd., Dalian, China). For normalization the gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used. Cycling conditions were as follows: initial denaturation at 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 15 s and 72 °C for 31 s, and no template controls were included for each assay. After PCR, a dissociation curve analysis was done. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method with MOCK cDNA from all samples as a reference. All experiments were performed in triplicate and repeated twice. All oligonucleotide primers (Table 1) were designed and synthesized by Takara (Dalian, China).

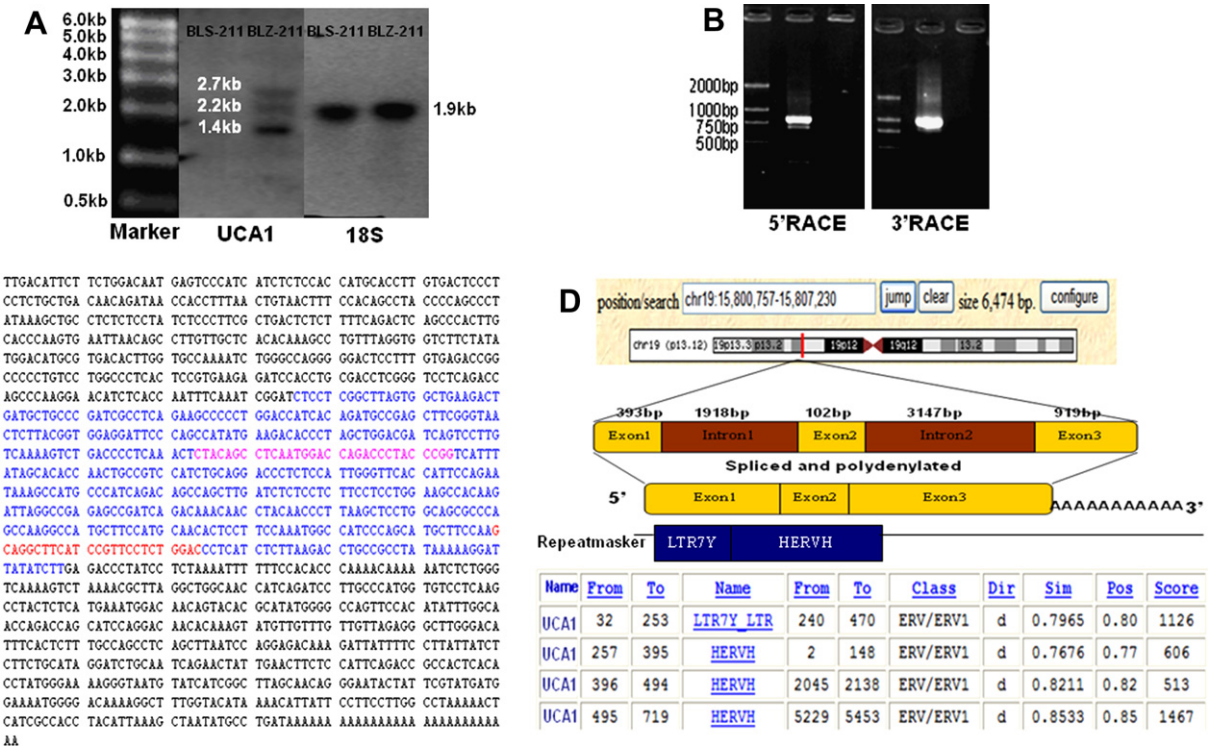
2.10. Statistical analyses

All statistical analyses were performed using the SPSS13.0 software. The results were presented as means ± S.E. Differences between means were analyzed using the unpaired Student's *t*-test (two-tailed). The probability value $P < 0.05$ was considered statistically significant.

3. Results

3.1. Molecular cloning and sequence analysis of UCA1 – a non-protein-coding RNA

Northern blotting analysis identified among several splice forms, a major UCA1 transcript approximately 1400 bp in length (Fig. 1A). UCA1 RNA was only expressed in BLZ-211 cells, no signal was observed in BLS-211 cells (Fig. 1A), which was consistent with our SSH data [10]. The 3′-cDNA end and 5′-cDNA end were confirmed to be 534 bp and 395 bp in length by RACE, respectively (Fig. 1B and C). The cloned full-length cDNA of UCA1 gene was 1442 bp (Fig. 1C), which was consistent with the result of Northern hybridization. UCA1 gene was detected in BLZ-211 cells but not in BLS-211 cells. This might explain the higher invasive ability of BLZ-211 cells [13]. Genomic sequence analysis revealed that this transcript was spliced and polydenylated and contained a polyadenylation signal (ATTAAA) located 16–21nt from the polyA tail. The UCA1 cDNA was found to have 100% identity with the human part of the DNA sequence of the chromosome 19 cosmid (AC004510), 99% identity with part sequence of human hypothetical LOC729642 mRNA (XM_001133784.1), and 97% identity with Homo sapiens cDNA FLJ35082 fis, clone PLACE6005351 (AK092401.1). No significant similarities were observed between UCA1 and other human known functional genes in Genebank nucleotide sequence databases. Moreover, no significant similarities were found between UCA1 transcript and mouse genomic and transcript in Genebank. Further analysis the Blast Tree View (http://www.ncbi.nlm.nih.gov/blast/treeview/blast_tree_view.cgi?; Database: nr, etc.) of UCA1 only identified UCA1 orththolegues in primates, including chimpanzee (*Pan troglodytes*, XM_001159928.1), Sumatran orangutan (*Pongo abelii*, AC210534.3), and rhesus monkey (*Macaca mulatta*, AC197808.1). No homologues were found in other species, such as rat, mouse, dog, cow, chicken, Drosophila melanogaster, honey bee, and the plant *Arabidopsis thaliana,* etc. in search



Name	From	To	Name	From	To	Class	Dir	Sim	Pos	Score
UCA1	32	253	LTR7Y_LTR	240	470	ERV/ERV1	d	0.7965	0.80	1126
UCA1	257	395	HERVH	2	148	ERV/ERV1	d	0.7676	0.77	606
UCA1	396	494	HERVH	2045	2138	ERV/ERV1	d	0.8211	0.82	513
UCA1	495	719	HERVH	5229	5453	ERV/ERV1	d	0.8533	0.85	1467

Fig. 1. Identification of *UCA1* full-length cDNA and gene structure. (A) Northern blot analysis of *UCA1* transcripts in BLS-211 and BLZ-211 cells. There were three different splice variants of UCA1 transcript in BLZ-211 cells, 1400 bp 2200 bp and 2700 bp, respectively, and the 1400 bp transcript was most abundant. The 18S rRNA was detected as control by hybridization with a 252bp cDNA probe and demonstrated equal loading of total RNA in each lane. (B) 5′ and 3′ ends of *UCA1* were extended from BLZ-211 cells using RACE technique. (C) The full-length cDNA sequence of *UCA1* gene. Blue part (including red and purple) represents EST sequence and the probe for Northern blotting. Red letters was the reverse complement sequence of 5′ RACE GSP1, purple letters was the 3′ RACE GSP2. (D) The *UCA1* gene was mapped to human chromosome 19p13.12 positive strand. UCA1 gene consists of three exons, the first two exons mainly consist a possibly nested LTR element of the ERV1 families (LTR7Y and HERVH).

of the Genbank database, suggesting that UCA1 was evolutionary conserved in some primates. The full-length cDNA was mapped to 19p13.12 positive strand with three exons (Fig 1D). Interestingly, the first two exons of UCA1 overlapped with a possibly nested LTR (long terminal repeat) element of the ERV1 family (LTR7Y and HERVH, Fig 1D), indicating the integration of a retrotransposon at this chromosomal location. Multiple stop condons and multiple short predicted ORFs (<240 nt) were found in all three frames of this transcript (Fig. 2A). None of the ORFs of UCA1 was conserved between human and other primates. The 5′ ends of all ORFs lacked an adequate Kozak start context [14]. Moreover, the deduced amino acid sequences of these ORFs do not contain any known protein motif nor do they share sequence similarities to other protein in any species. The TestCode value for this sequence was 0.4367, indicating that it was probably a ncRNA. To find out whether the predicted short ORFs were capable of translating into corresponding short peptides in human cells, all five putative ORF sequences as well as the full-length UCA1 cDNA were amplified and subcloned separately into the mammalian expression vector pEGFP-N3. The constructs were then transfected into BLS-211 cells to be expressed as fusion proteins but no fluorescence in BLS-211 cells transfected by p*UCA1*-EGFP construct (Fig. 2B). Taken together, these data demonstrated that UCA1 is a ncRNA.

3.2. Spatial and temporal expression patterns of *UCA1*

RT-PCR analysis of spatial and temporal expression patterns of *UCA1* in various phase embryo tissues, adult tissues, and bladder cancer tissues revealed that high expression of UCA1 gene begins at early stage after fertilization and continues during embryonic development (Fig. 2C1). In tissues of 28 week pregnancy, UCA1 expression levels differed in different organs. It was up-regulated in heart, urinary bladder, and uterus, but lower in liver, kidney, lung, spleen, intestine, stomach, skin, and cervix (Fig. 2C2). In adult, the expression of UCA1 was turned off in most tissues. It was only expressed in heart, spleen, and placental tissue (Fig. 2C3). The differential expression of UCA1 between human normal and cancerous tissues was further demonstrated in different cancer tissues. In urinary system, it was up-regulated in bladder cancer tissues, but no expression in normal bladder tissues (Fig. 2C4), and no expression in renal cancer tissues, normal renal tissues and hyperplasia of prostate gland tissues (date not shown). In other common tumors, UCA1 was up-regulated in tumor tissues, such as colon, cervix, lung, thyroid, liver, mammary gland, esophagus and stomach, compared with corresponding non-cancerous tissues (data not shown). The differential in UCA1 expression between human embryonic tissues, normal adult tissues and cancer tissues suggested that UCA1,

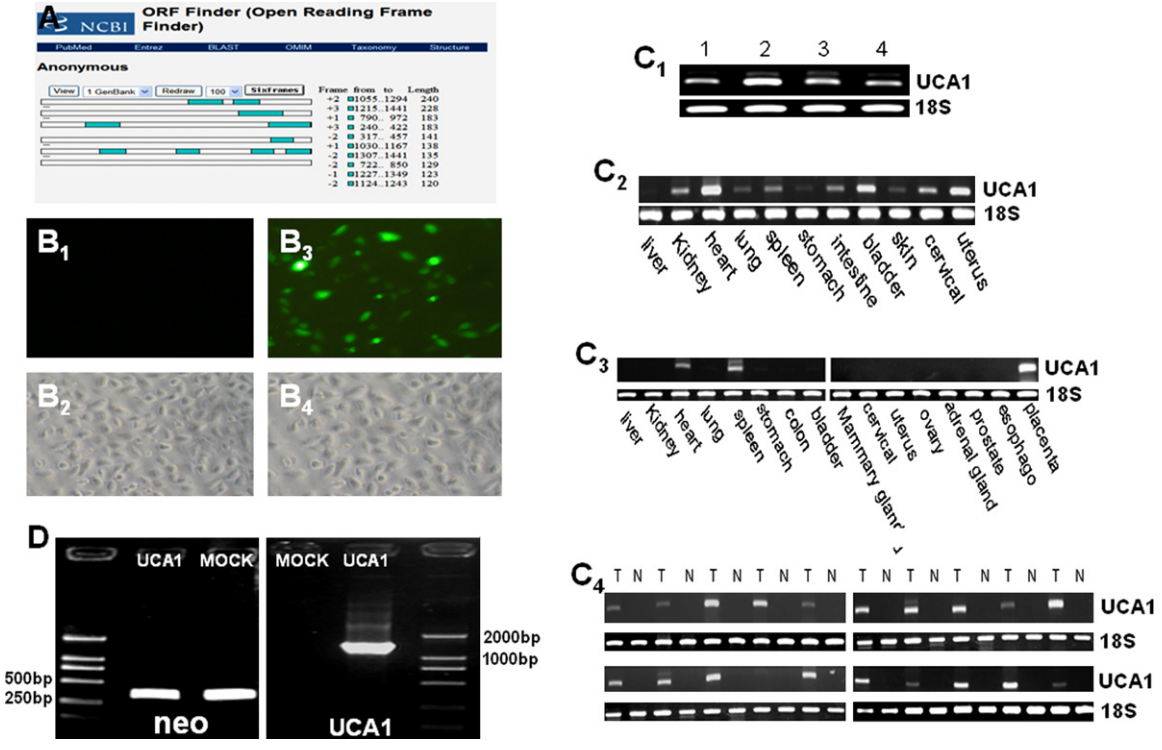


Fig. 2. (A) Analysis the open reading frames (ORF) of *UCA1*. (B) Coding ability assay of *UCA1* in vitro. (B1, B3) Green fluorescent image of BLS-211 cells transiently transfected with the *pUCA1*-EGFP plasmid and pEGFP-N3 positive control plasmid, respectively. (B2, B4) Bright field microscopic image corresponding to B1 and B3, respectively. (C) Tissue expression patterns of *UCA1* RNA. (C1) *UCA1 RNA* expression in human normal embryonic tissue. Lanes 1–4: the gestational weeks were five, ten, seven, eight, respectively. (C2) *UCA1* expression in tissues of 28 week pregnancy. (C3) Expression of *UCA1* RNA in 16 normal adult tissues. (C4) *UCA1* expression in 20 different human bladder TCC tissues (T) and 20 different normal bladder tissues (N). The level of 18S rRNA in each sample was also measured as control and did not demonstrate deviation among the samples. (D) Positive clone of pcDNA/*UCA1* stable transfection in BLS-211cells was identified by RT-PCR for *UCA1* and neo gene expression. Left: neo gene was amplified from pcDNA/*UCA1* transfected cells and pcDNA3.1 transfected cells. Right: the *UCA1* sequence was only amplified from pcDNA/*UCA1* transfected cells, no products in pcDNA3.1 transfected cells (MOCK).

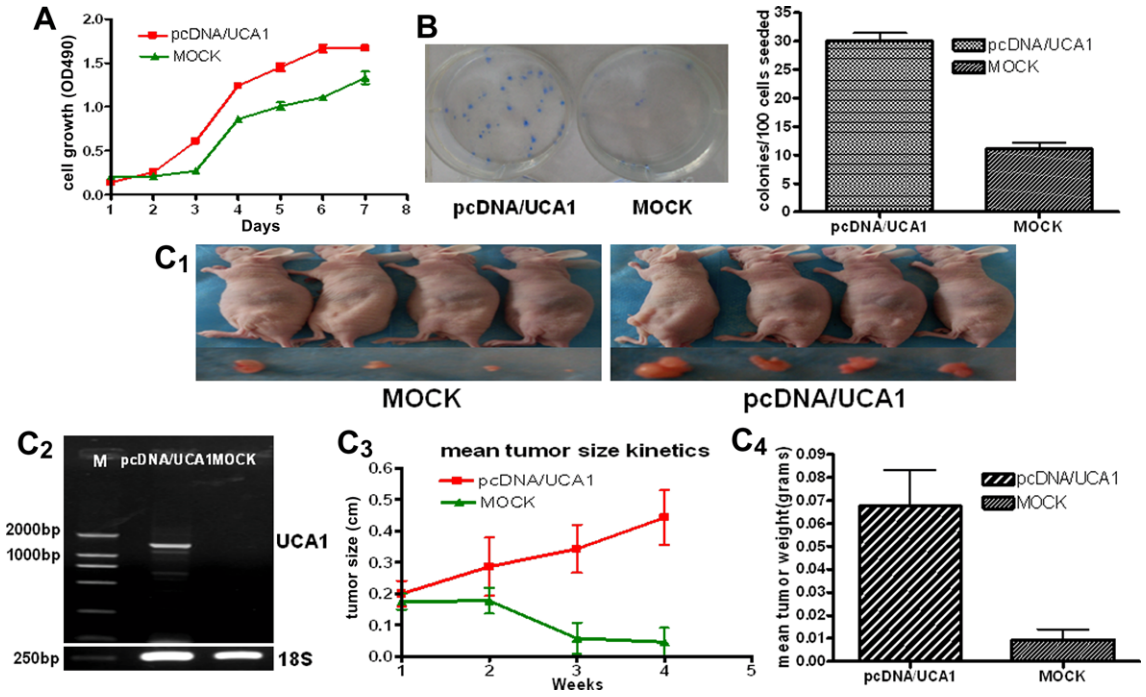


Fig. 3. UCA1 expression promoted proliferation potential of BLS-211 cells in vitro and in vivo. (A) MTT assay was used to assess the effect of *UCA1* expression on growth of BLS-211 cells in vitro. Results represented the mean of three independent experiments performed in triplicates. The promotion effect became obvious from the fifth day, and the difference was significant ($P < 0.01$). (B) Colony formation assay. Representative cultures were shown, the colonies that pcDNA/*UCA1* formed was 2.7 times than MOCK, difference was significant ($P < 0.05$). (C) *UCA1* expression enhances the tumorigenic potential of bladder carcinoma cells in vivo. (C1) Exposure of their tumors when mice were killed. (C2) RT-PCR detected *UCA1* expression in tumor tissues of nude mice. The UCA1 RNA was only amplified from the tumor tissue derived from pcDNA/*UCA1* transfected cells, no products were detected in tumor tissue from pcDNA3.1 transfected cells (MOCK). (C3) UCA1 expression persistently promoted tumor growth in vivo. The promotion effect became apparent from the second week post injection, and the difference was significant ($P < 0.01$). (C4) UCA1 expression significant increased of about seven times of mean tumor weights. The difference was significant ($P < 0.01$).

in addition to its role in embryonic development, might also be important in carcinogenesis.

3.3. Ectopic UCA1 expression enhances the tumorigenic potential of bladder carcinoma cells in vitro and in vivo

To investigate whether UCA1 expression plays a role in bladder cancer progression, we established the pcDNA/*UCA1* stable transfectant (Fig. 2D). The expression of UCA1 in BLS-211 cells indicated that it may have an important role for bladder tumor growth. Compared with MOCK transfectant, the stable UCA1 transfectant promoted BLS-211 cell proliferation as measured by MTT assay (Fig. 3A), and colony formation ability increased 2.7 times (Fig. 3B). We also identified that UCA1 expression promoted the tumorigenicity of BLS-211 cells in vivo. As shown in Fig. 3C1–4, tumors derived from the pcDNA/*UCA1* cells were significantly larger than those from the MOCK cells. Moreover, we found that the tumors of pcDNA/*UCA1* transfected cells persistently grew in size, but the tumors of MOCK transfected cells gradually diminished.

3.4. UCA1 expression increases the motility, invasion and drug resistance of BLS-211 cells

Ectopic expression of *UCA1* strongly promoted the motility and invasion ability of bladder TCC cell line BLS-211 cells (Fig. 4A). Moreover the expression UCA1 in BLS-211 cells could lead to resistance to cytotoxic drugs as measured by an MTT assay, such as cisplatin, its IC_{50} increased 2.4 times in UCA1 expressed BLS-211 cells compared with the MOCK transfectants (Fig. 4B), and mitomycin, the IC_{50} increased 1.9 times (data not shown).

3.5. UCA1 expression modulates the expression of several genes involved in tumorigenic potential, drug resistance and embryonic development

To obtain a first insight into a possible role of UCA1 in tumor progression and to further determine the different targets and pathways which are affected by UCA1 RNA, we detected that 16 genes were up-regulated (Table 2) and 26 genes were down-regulated (Table 3) by ectopic expression of the UCA1 RNA in BLS-211 cells through microarray assay. Results indicated that expression of UCA1 RNA regulated the expression of several gene products that mediated some aspects of the tumorigenic processes and/or associated with embryonic development (Tables 2 and 3). The changes of expression of the several representative genes were confirmed through real time PCR (Fig. 4C3–C5), the up-regulated genes including wingless-type MMTV integration site family, member 6 (WNT6) [15], CYP1A1 (cytochrome P450, 1A1) [16], and AURKC (aurora kinase C) [17], and the down-regulated genes including methyl-CpG binding domain protein 3 (MBD3) [18–20], and SR (serine/arginine-rich) protein-specific kinase 1 (SRPK1) [21,22], which were identical with the microarray results (Tables 2 and 3).

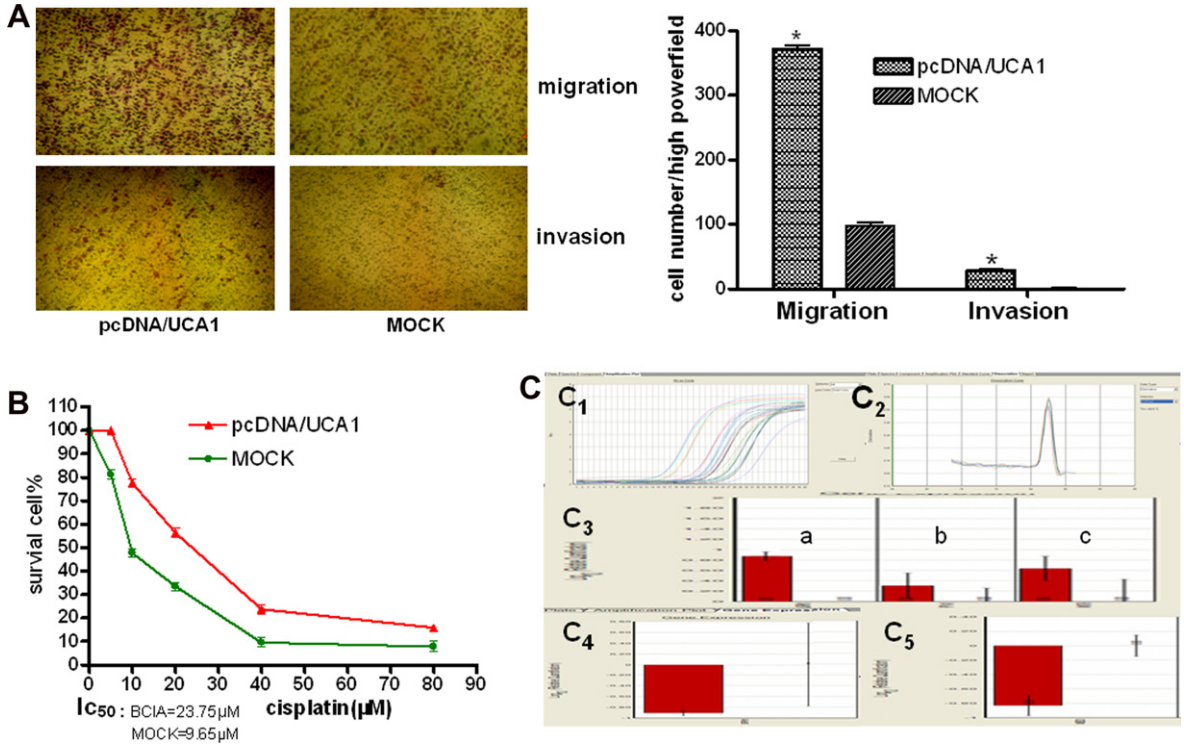


Fig. 4. (A) UCA1 expression increased the motility and invasive potential of BLS-211 cells in vitro. Representative visualfield of pcDNA/*UCA1* cells (left) compared with MOCK cells (right) was shown. Representative number of motility or invasion cells was counted under the microscope in 10 random fields at 400×. Significant difference from pcDNA3.1 control cells was indicated by asterisks ($P < 0.01$). (B) Cisplatin resistance assay. The IC_{50} increased 2.4 times in UCA1 expressed BLS-211 cells compared with the MOCK transfectants. (C) Verification microarray results through real-time PCR. (C1) Real-time amplification plots. (C2) Representative dissociation curves. (C3–C5) Real-time PCR analysis results: AURKC (C3a), CYP1A1 (C3b), WNT6 (C3c) mRNA expression were increased; SRPK1 (C4), and MBD3 (C5) mRNA expression were reduced in pcDNA/*UCA1* cells, with MOCK cDNA as a reference. GAPDH was used as an internal control.

Table 2
Genes induced at least twofolds by *UCA1* expression

GeneBank accession number	Gene symbol and name	Folds change	Functional category
NM_002949	Mitochondrial ribosomal protein L12	6.6	Mitochondrial translation
NM_000499[a]	Cytochrome P450 family 1, subfamily A	5.5	Metabolism
NM_004309	Rho GDP dissociation inhibitor alpha	3.0	Signal transduction
NM_001015878[a]	Aurora kinase C	4.7	Cell division/chromosome degregation
NM_005059	Relaxin 2	6.1	Extracellular matrix remodel/signal transduction/ pregnancy/hormone activity
NM_001386[a]	Legumain	6.5	Extracellular matrix remodel
NM_031431	Component of oligomeric golgi complex 3	5.9	Protein glycosylation/Golgi structure and funct
NM_014208	Dentin sialoposphoprotein	8.8	Tooth development/nucleolus
NM_153221	Cartilage intermediate layer protein 2	4.2	Endochondrial bone development
NM_001004051	G protein-coupled receptor associated sorting protein 2	7.8	Membrane receptor degradation
NM_003884	p300/CBP-associated factor	5.4	Transcriptional regulator
NM_006522[a,b]	Wingless type MMTV integration site family,member 6	4.8	Wnt signaling pathyway
NM_016350[b]	Ninein (GSK3B interacting protein)	4.9	Centrosome associated protein
NM_018386	FLJ11305	6.0	Unknown
A_23_P75197	A_23_P75197	6.9	Unknown
A_23_P16408	A_23_P16408	6.3	Unknown

[a]Genes that have been reported to mediate some aspects of the tumorigenic processes.
[b]Genes associated with embryonic development.

4. Discussion

UCA1 is believed to exert its functions as a ncRNA in the regulation of embryonic development and bladder cancer invasion and progression. The key feature of all ncRNA is that they are not translated into proteins, but rather function directly at the RNA level [23–25]. Moreover, the RIKEN standard in Tokyo classifies the transcript as ncRNA if the sequence of the putative ORF of the transcript is <300 base pairs (bp) [26]. There are five putative ORFs in the 1442 bp

Table 3
Genes reduced at least twofolds by *UCA1* expression

GeneBank accession number	Gene symbol and name	Folds change	Functional category
NM_003926[a,b]	Methyl-CpG binding domain protein 3	3.9	Transcriptional repressor/pregnancy
NM_018957	SH3-domain binding protein 1	5.1	Cell signaling transduction
NM_001622	Alpha-2-HS-glycorprotein	3.1	Skeletal development/negative acute phase response
NM_001009991	Synaptotagmin-like 3	4.1	Exocytosis
ENST00000227451[b]	Deltex 4 homology (drosophila)	3.5	Notch signaling pathway/ubiquitin-protein lignase activity
NM_00337[a]	SFRS protein kinase 1	5.9	pre-mRNA splicing/cisplatin sensitivity
NM_002862	Phosphorylase, glycogen, brain	3.7	Metabolism
NM_018796	Solute carrier family 38, member 2	3.8	Amino acid transporter
ENST00000327926	Olfactory receptor, family 5, subfamily AS, member 1	4.7	Antigen binding/immune response
NM_007152	Zinc finger protein 195	7.3	Transcription regulator
NM_138330	Zinc finger protein 675	3.0	Transcription regulator
NM_199511[a]	Steroid sensitive gene 1	7.8	Apoptosis
NM_139322	Attractin	2.0	Development/inflammatory response
NM_016199[a]	LSM7 homolog, U6 small nuclear RNA associated (*Saccharomyces cerevisiae*)	4.5	pre-mRNA splicing factor
NM_021643[a]	Tribbles homolog 2 (Drosophila)	3.3	Slow cell cycle
NM_002197[a]	Aconitase 1, soluble	3.1	Metabolism/regulation of translational initiation by iro
AK056670	Solute carrier family 38, member 1	3.7	Amino acid transporter
NM_080608	Chromosome 20 open reading frame 165	4.4	Unknown
NM_001001921	OR5AS1	4.5	Unknown
NM_030891	Leucine rich repeat containing 3	3.3	Unknown
NM_152779	Hypothetical protein MGC26856	8.2	Unknown
NM_152761	Hypothetical protein FLJ25444	5.0	Unknown
NM_152510	HORMA domain containing 2	4.3	Unknown
NM_006383	Calcium and integrin binding family member 2	3.0	Unknown
A_23_P86762	Immunoglobulin binding protein 2	9.9	Unknown
NM_198991	Potassium channel tetramerisation domain containing 1	3.3	Unknown

[a]Genes that have been reported to mediate some aspects of the tumorigenic processes.
[b]Genes associated with embryonic development.

UCA1 transcript, and their sequences are all <300 bp. Furthermore, the deduced amino acid sequences of these ORFs do not contain any known protein motif nor do they share similarities to other proteins in any species. So far, only two human endogenous retrovirus (HERV) families, HERV-K and HERV-W, which contained intact ORFs and could encode functional proteins have been reported [27,28], most other HERV sequences are protein-coding deficient because they accumulated a variety of mutations and deletions or due to homologous recombination between 5′- and 3′-LTRs and are present only as solitary LTRs in the genome [29,30]. Moreover, the proteins supposedly encoded by these ORF sequences of UCA1 were not detected in cells after transfection with these sequences. All of the results supported that this transcript might function as a ncRNA.

Evidence for the origin of the UCA1 gene was based on the finding that the first two exons of UCA1 sequence mainly consist of a possibly nested LTR element of the ERV1 family (LTR7Y and HERVH), indicating that a retrotransposon integrated at this chromosomal site. Another ncRNA, highly up-regulated in liver cancer (HULC), associated with hepatocellular carcinoma, is similar to the UCA1 gene, in that the first exon of HULC gene overlaps with an LTR of the mammalian LTR transposon1A (MLT1A) type [5]. Interestingly, the HERV-derived sequence in UCA1 RNA was only conserved in certain primates, no homologue to other species. So far, whether the HERVH-derived sequences could encode proteins is unclear, however, HERVH-derived transcripts have been observed in a variety of cell lines [31–34]. Although the precise roles of HERVs in carcinogenic process have not been elucidated, several studies have implicated the possible involvement of HERVs in malignancy [34–38].

The potential roles of UCA1 in embryonic development and bladder carcinoma progression were based on its striking expression in embryonic tissues and bladder TCC tissues. During development, UCA1 was transcribed at high levels in placenta, embryo, and most of fetal tissues, but after birth its expression was turned off in most tissues. Then, it was reactivated during adult tumorigenesis. The spatial and temporal expression patterns of UCA1 was similar to the expression patterns of ncRNA H19 [39], indicated that it was an oncofetal gene. Especially, exogenous expression of UCA1 enhanced tumorigenicity, invasive potential and drug resistance in BLS-211 cells, which suggested that UCA1 RNA might play some roles in bladder cancer invasion and progression.

The ncRNAs might interact with its target gene/protein and then through some still unidentified pathways regulate the expression of effector genes. In microarray assays, we identified several genes significantly up- and down-regulated when exogenously expressing UCA1 in BLS-211 cells. Some of which have already been described in the context of different cancers, such as WNT6, CYP1A1, AURKC, etc. Some were associated with embryonic development, such as MBD3, WNT6, etc. We speculated that UCA1 promoted tumor progression and embryonic development mediated via these potential target genes. The regulatory role of UCA1 in cisplatin resistance was believed to be associated with the expression suppression of SRPK1 gene. SRPK1 is a highly specific protein kinase for the SR family of splicing factors [40]. It has been identified as a cisplatin sensitivity gene, inactivation of this gene in human ovarian carcinoma cells fourfold resistance to cisplatin

[21]. Nevertheless, the exact role of UCA1 in embryonic development, bladder cancer invasion and progression is not clear, detailed understanding will require further investigation.

In this study, we characterized a non-protein-coding RNA, UCA1, from human bladder TCC cell line BLZ-211 cells. The temporal and spatial expression patterns of UCA1 suggest that it might be an oncofetal gene. In the urinary system, it is highly specific to bladder cancer. Our results indicate that UCA1 is expressed during embryonic development and bladder cancer. The cispltin resistance function is potentially related to its regulatory roles in SRPK1. Result from present study supported that UCA1 might be a promising biomarker for bladder cancer invasion and progression, and could also be a potential therapeutic target in bladder cancer.

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