Increased urothelial cancer associated 1 is associated with tumor proliferation and metastasis and predicts poor prognosis in colorectal cancer

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Abstract. Long non-coding RNA, urothelial cancer associated 1 (*UCA1*), is reported to play a critical role in progression of carcinogenesis. In the present study, we identified differential expression of *UCA1* in colorectal cancer (CRC) and paired peritumoral tissues using gene expression microarray analyses. qPCR analysis confirmed that *UCA1* was upregulated in CRC (P<0.001) and the expression of *UCA1* was statistically correlated with lymph node metastasis (P=0.040), distant metastasis (P=0.043) and tumor stage (P=0.010). Kaplan-Meier analysis indicated that patients with high *UCA1* expression had a poor prognosis. Moreover, multivariate analysis identified *UCA1* overexpression as an independent predictor for CRC. We also found that knockdown of *UCA1* significantly suppressed cell proliferation and metastasis in CRC cells. Flow cytometry assays showed *UCA1* silencing induced G0/G1 growth arrest and apoptosis of CRC cells. To further investigate the regulatory mechanisms of *UCA1*, we identified that Ets-2 bound to the *UCA1* core promoter using luciferase assays. Collectively, our findings suggested that *UCA1* might be an important prognostic indicator in CRC and may be a potential target for diagnosis and gene therapy.

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Abbreviations: CRC, colorectal cancer; UCA1, urothelial carcer associated 1; LncRNA, long non-coding RNA; qPCR, quantitative real-time polymerase chain reaction; sncRNA, small non-coding RNAs; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RTCA, real-time cellular analysis; FACS, fluorescence-activated cell sorting

Key words: colorectal cancer metastasis, long non-coding RNAs, microarray, *UCA1*, prognosis

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer in males and the second most frequently diagnosed cancer in females (1). Although current radio-chemical therapies and surgery have shown great progress, the morbidity and mortality rate of CRC has still increased over the years (2). Therefore, there is an urgent need to understand the molecular mechanisms underlying CRC tumorigenesis and to identify new therapeutic targets for CRC.

With advances in sequencing technologies, non-coding RNAs, which account for 70% of the human genome, have shown great potential in biological research and clinical diagnostics. Non-coding RNAs (3) are divided into two major classes: small non-coding RNAs (sncRNAs) and long non-coding RNAs (lncRNAs), based on the transcript size. Although many studies proved that sncRNAs, especially microRNAs (miRNAs) (4,5), played important roles in the pathogenesis of many diseases, little is known about lncRNAs. LncRNAs (>200 nucleotides), which were once regarded as 'transcriptional noise' in the genomic RNA, have been proved to play important roles in regulating gene expression at the epigenetic, transcriptional and post-transcriptional levels (6,7). In the last few decades, a large number of lncRNAs [e.g., H19 (8,9), MALAT1 (10,11) and HOTAIR (12)] have been identified and studied in a variety of diseases. It is expected that lncRNAs could be used in clinical applications as prognostic or predictors of cancer. In CRC, emerging evidence (13-15) revealed that aberrant expression of particular lncRNAs could represent novel cancer biomarkers. PVT-1 (16), generates anti-apoptotic activity in CRC, was a prognostic indicator for CRC patients. LncRNA 91H (17) was considered as a prognosis indicator that contributed to tumor metastasis and predicted patient survival in CRC. However, the relationship between lncRNA expression level and progression of CRC is still elusive.

To screen tumor initiation and progression-associated lncRNAs in CRC, we profiled the expression of lncRNAs in six pairs of CRC tissues and peritumoral tissues using microarray analysis. We found that the levels of *UCA1* in CRC tissues were 7.104 times higher than peritumoral tissues. *UCA1*, which was identified as a novel bladder transitional cell carcinoma (TCC) transcript (18), played a key role in cellular proliferation, metastasis and oncogenesis, and was identified as a novel therapeutic target (19,20). However, the role of *UCA1* in CRC is not well studied, therefore, we focused our attention on *UCA1* and investigated the clinical values and biological roles of *UCA1* in CRC.

Materials and methods

Study subjects and sample collection. The Institutional Review Board of Sun Yat-Sen University approved the study protocol. In the present study, 54 CRC samples and paired peritumoral samples, deposited between 2010 March to 2010 July in Tissue bank of the Sixth Affiliated Hospital, Sun Yat-Sen University, were used. Informed consent was obtained from participants for the use of their tissues in the present study. All the diagnoses of CRC were histopathologically confirmed. The data of clinicopathological characteristics (include age, gender, tumor size, tumor location, differentiation, histological stage, tumor invasion and lymph node metastasis) were collected from medical records and pathology reports. The stage of CRC was evaluated based on the American Joint Committee on Cancer staging Manual. Follow-up was performed according to the National Comprehensive Cancer Network (NCCN) guidelines.

Cell lines and cell culture. All the human CRC cell lines (HCT116, SW480, RKO, HCT8, LoVo, T84, HT29, DLD1, HCT15, Colo205 and Caco2) were purchased in March 2013 from the Culture Collection of Chinese Academy of Science, Shanghai, China. They were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) or RPMI-1640 medium, supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS; Life Technologies, Carlsbad, CA, USA). The cells were grown at 37˚C with 5% CO_2 in a humidified incubator.

Microarray and computational analysis. Six CRC tissues and paired peritumoral tissues were used to investigate the expression of both protein coding mRNAs (~26109) and lncRNAs (~30586) using the Human 8x60K LncRNA Microarray V3.0 (Arraystar, Rockville, MD, USA). The raw signal intensities were normalized and hierarchical clustering of differentially expressed lncRNAs was performed using GeneSpring GX v11.5.1 software (Agilent Technologies, Santa Clara, CA, USA). Kangcheng Biology Engineering Co., Ltd., (Shanghai, China) performed the microarray analysis.

siRNA transfection. HCT116 and DLD1 cells were transfected with siRNA oligonucleotides using the Lipofectamine RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA, USA) for 48 h in 6-well plates. Both the *UCA1* siRNA and the scrambled control siRNA were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). Each well contained $3x10^5$ cells, 5 μl siRNA, 5 μl Lipofectamine RNAiMAX and 500 μl Opti-MEM (Invitrogen). Both the *UCA1* siRNA and the scrambled control siRNA were synthesized by Guangzhou Ribobio. We selected one of the siRNA sequences from three candidates based on the highest knockdown efficiency, as confirmed by qPCR. The sequences of these siRNAs are listed in Table I.

Reverse transcription and quantitative real-time PCR (qPCR). RNAs from cells and tissues were extracted by using the TRIzol reagent (Invitrogen), according to the manufacturer's instructions. Reverse transcription was carried out with ReverTra Ace qPCR RT Master Mix with gDNA remover (Toyobo Co., Ltd., Osaka, Japan). qPCR was conducted using SYBR-Green real-time PCR Master Mixes (Applied Biosystems, Foster City, CA, USA), using the following conditions: 95˚C for 10 min; and 40 cycles of 95˚C for 15 sec; 60˚C for 1 min. The PCR products were subjected to 1% agarose gel and the relative *UCA1* expression levels were quantified by using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA). In each qPCR assay, amplification of the housekeeping gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed as the internal control. qPCR was performed in triplicate, including no-template controls. The amplification of the appropriate product was confirmed by melting curve analysis following amplification. The relative expression of *UCA1* was calculated using the comparative cycle threshold (CT) ($2^{-\Delta\Delta CT}$) method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control to normalize the data. The PCR primers used in the present study are listed in Table I.

Cell proliferation assays. Cell proliferation was performed on the xCELLigence real-time cellular analysis DP device (ACEA Biosciences, San Diego, CA, USA). Cells were seeded in cell culture E-plates at a density of 6000 cells/well and incubated at 37˚C and 5% CO_2. The plate was automatically monitored and recorded every 15 min for a total of 120 h. Three replicate cells were used and three independent experiments were conducted. The results were expressed as the parameter 'Cell Index'.

Cell migration and invasion assays. Cell invasion assays were performed using a 24-Multiwell insert plate with an 8.0-micron pore size membrane (BD Biosciences, Bedford, MA, USA) chamber containing a Matrigel-coated membrane. Cells were prepared by suspending them in serum-free DMEM and adding them to apical chambers whose reverse sides had been covered by fibronectin (BD Biosciences). Cells at a density of 40,000 cells/100 μl of DMEM media were placed into the upper chamber of the Transwell plate and 700 μl of DMEM medium containing 20% FBS was to the basal chambers for better cell access. After incubating the plate for 48 h at 37˚C, 5% CO_2 atmosphere, invasive cells were stained with DAPI and counted using a fluorescence microscope. The cell migration assay was the same but without Matrigel. Experiments were repeated independently three times.

Cell cycle and apoptosis assays. Cells were stained by using cell cycle staining solution (Lianke, Hangzhou, China) and analyzed by flow cytometry. Approximately $2x10^5$ cells were

Table I. Primers used in the present study.

Gene	Sequence (5'-3')	Experimental use
UCA1 siRNA	GCAUCCAGGACAACACAAAdTdT dTdTCGUAGGUCCUGUUGUGUUU	siRNA
UCA1 mRNA	CTCTCCATTGGGTTCACCATTC GCGGCAGGTCTTAAGAGATGAG	qRCR/RT-PCR
GAPDH mRNA	GACAGTCAGCCGCATCTTCTT AATCCGTTGACTCCGACCTTC	qRCR/RT-PCR
UCA1 promoter	CGGGGTACCTCCTAAGGGGTTTCCTTT CCCAAGCTTGGCTGTTAATTCACTTGGG	PCR
Ets-2 binding site	AGATCTAAATGACCCAGGAGCTGATA AGAGGACAGCCTGAGATGTGCCTGTGG	Site-directed mutagenesis
CEBP binding site	CTCAAGTTAGGGAACTGTCAGGCCTCTGA CGTTACAGGGTGATGTGACCTCAGCCCAC	Site-directed mutagenesis
C-myb binding site	CGCCTGAAACCCAGGACCAGGAAAAGA TGCCTGGGGCTCATCTGAGATGCCCAC	Site-directed mutagenesis
GATA1 binding site	AGCGAGAGGGTGGGCTGAGGTCACATC TTTTCCTGGTCCTGGGTTTCAGAACTG	Site-directed mutagenesis
CREB/α binding site	GAGCTCCACATCACCCTGTAACGTTTCC GCCCACCCTCTTATCTTTTCCTGGTCCT	Site-directed mutagenesis

pelleted by centrifugation and washed with PBS. Cold 75% ethanol (1 ml) was added to the cells at -20˚C overnight. The ethanol was discarded and PBS was added to rehydrate the cells for 15 min. The cells were incubated with 1 ml DNA staining solution at room temperature, then sorted by FACSCalibur (BD Biosciences, Flanklin Lakes, NJ, USA). The results were analyzed using ModFit 3.0 software (Verity Software House, Topsham, ME, USA). Cell apoptosis was detected using an Annexin V/PI apoptosis kit (Lianke). Cells ($2x10^5$) were collected by centrifugation and resuspended in 500 μl of 1X binding buffer. Annexin V-FITC (5 μl) and 10 μl propidium iodide were added and the cells were incubated for 5 min in the dark, at room temperature before being examined using flow cytometry.

Plasmid constructs and site-directed mutagenesis. The *UCA1* promoter (-500 to +200 bp) was obtained by PCR (PrimeSTAR Max DNA polymerase; Takara, Shiga, Japan). The PCR products were subjected to 1% agarose gel and isolated from the gel using a Gel/PCR Extraction kit (Biomiga, San Diego, CA, USA). The pGL3-*UCA1*-promoter vector was constructed from the pGL3 basic vector and the purified *UCA1* promoter using Ligation high (Toyobo). Site-directed mutagenesis was performed using a KOD-plus-mutagenesis kit (Toyobo), according to the manufacturer's instructions. The pGL3-*UCA1*-promoter vector was used as the template. The mutation sites were designed at the 3' region of the primers. All the primers are listed in Table I.

Luciferase assays. Lipofectamine 3000 (Invitrogen) was used to co-transfect CRC cells with the pGL3-*UCA1*-promoter (mutation), in combination with the pRL *Renilla* luciferase reporter vectors (Promega, Madison, WI, USA) as an internal control reporter. After incubating cells for 48 h at 37˚C, luciferase activities were detected using a Dual-luciferase reporter assay (Promega), according to the manufacturer's instructions. Firefly luciferase luminescence was measured using Varioskan Flash (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis. Statistical analyses were performed with the SPSS statistical package (version 16.0; SPSS, Inc., Chicago, IL, USA). Data are presented as means ± standard deviation (SD). Differences between groups were compared using paired t-tests, unpaired Student's t-tests or the Mann-Whitney U test. Overall survival curves were plotted according to the Kaplan-Meier method, with the log-rank test applied for comparison. A Cox proportional hazards model univariate and multivariate analysis were performed to evaluate the association between *UCA1* expression and clinicopathological parameters on the overall survival. In all analyses, a probability (P) of ≤0.05 was considered statistically significant.

Results

UCA1 is highly expressed in CRC tissues and cell lines. To determine the effects of lncRNAs on CRC, we profiled the expression of lncRNAs in six CRC tissues and paired peritumoral tissues using microarray analysis. Hierarchical clustering showed systematic variations in the expression of lncRNAs (fold-change ≥4) between CRC and paired peritumoral tissues (Fig. 1A). We noted that *UCA1* was remarkably upregulated (fold-change, 7.104, P=0.0066) in CRC according

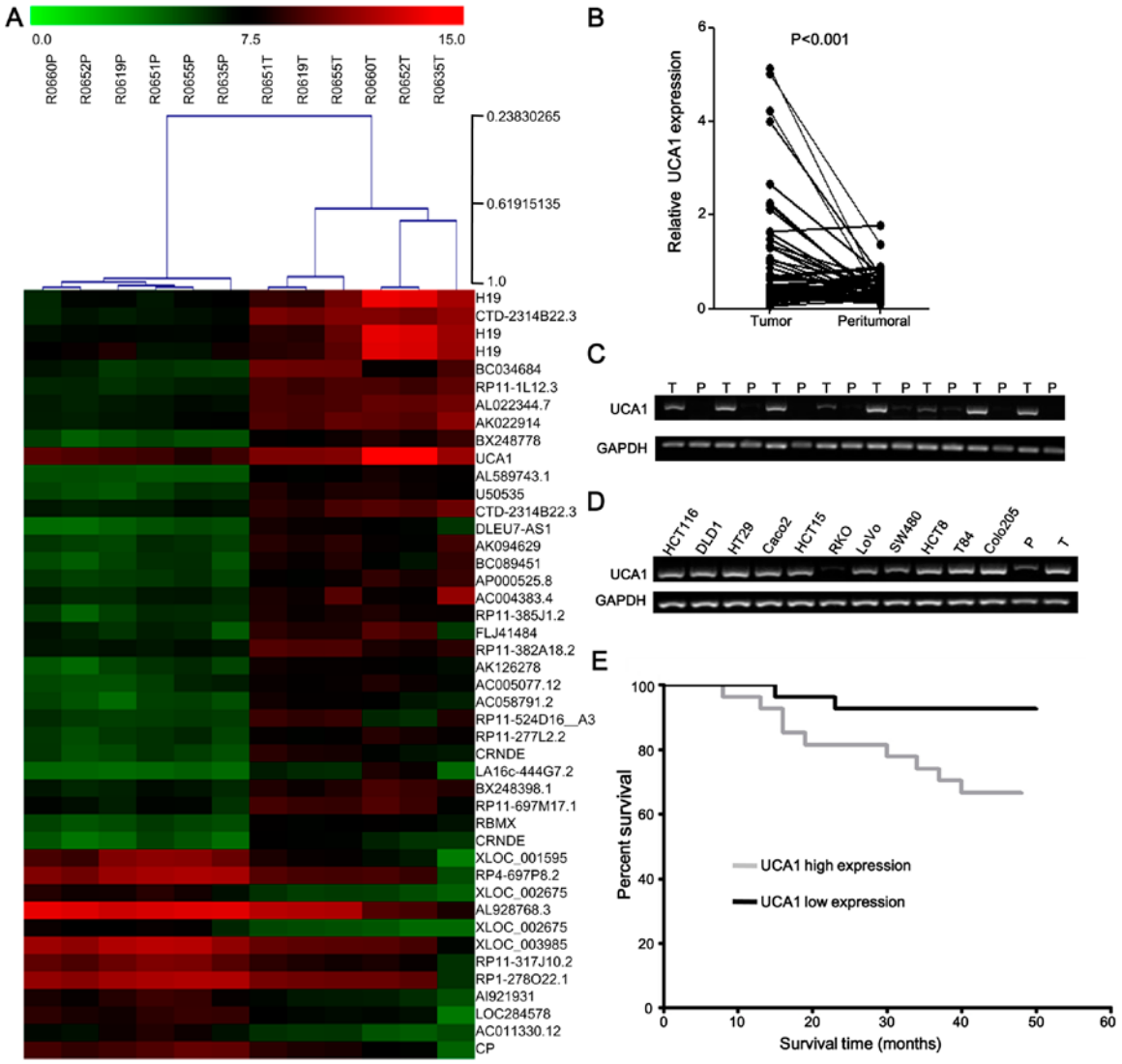

Figure 1. Expression of *UCA1* in CRC tissues and cell lines. (A) Hierarchical clustering showed systematic variations in the expression of lncRNAs (fold-change ≥4) between CRC and paired peritumoral tissues. The red and green indicate high and low expression, respectively. (B) qPCR analyses of *UCA1* expression levels in tumor tissues and peritumoral tissues from 54 pairs of CRC samples. (C) Representative results for expression of *UCA1* in tumor tissues (T) and peritumoral tissues (P). *UCA1* levels were normalized to that of GAPDH. (D) PCR analyses of *UCA1* expression levels in 11 CRC cell lines (HCT116, SW480, RKO, HCT8, LoVo, T84, HT29, DLD1, HCT15, Colo205 and Caco2 cells). The CRC tissue and peritumoral tissue as the positive control and the negative control, respectively. (E) Kaplan-Meier overall survival curves for 54 patients with CRC classified according to *UCA1* expression level. The overall survival of the high *UCA1* expression group (n=27) was significantly higher than that of the low expression group (n=27; log rank test; P=0.021).

to microarray data. To validate the microarray analysis findings, the expression of *UCA1* was detected by qPCR from 54 pairs of CRC tissues compared with paired peritumoral tissues. The results showed that *UCA1* expression was significantly higher in the tumor tissues than in the paired peritumoral tissues (P<0.001) (Fig. 1B and C). PCR assays were further developed to quantify the *UCA1* expression in 11 CRC cell lines (HCT116, SW480, RKO, HCT8, LoVo, T84, HT29, DLD1, HCT15, Colo205 and Caco2 cells). Almost all the cell lines showed positive, high expression, except RKO (Fig. 1D). These results revealed that the expression of *UCA1* is upregulated in CRC tissues.

UCA1 is a predictor of poor outcome in patients with CRC. To assess the correlation of *UCA1* expression with clinicopathological characteristics, the expression levels of *UCA1* in CRC tissues were categorized as low or high in relation to the median value. The clinicopathological characteristics are summarized in Table II. *UCA1* expression in CRC was significantly correlated with lymphatic metastasis (P=0.040), distant metastasis (P=0.043) and tumor stage (P=0.010). However, there was no significant association between *UCA1* expression and age, gender, differentiation, lymphatic invasion or venous invasion (all P>0.05). With regard to overall survival, patients with high *UCA1* expression had a significantly poorer prognosis than those with low *UCA1* expression (Log-rank P=0.021) (Fig. 1E). Unvariate analysis of overall survival revealed that the relative level of *UCA1* expression (P=0.038), lymphatic invasion (P=0.001), lymph node metastasis (P=0.015), distant metastasis (P=0.001) and tumor stage (P=0.002) were prognostic indicators (Table III). The other clinicopathological characteristics, such as age, gender, histological grade, venous invasion, were not statistically significant prognosis factors (P>0.05; Table III). Multivariate

Table II. *UCA1* expression and clinicopathological factors in 54 CRC cases.

Factors	High expression (N=27) N (%)	Low expression (N=27) N (%)	P-value
Age (years)			
≤60	9 (33.3)	10 (37.0)	0.776
>60	18 (66.7)	17 (63.0)	
Gender			
Male	17 (63.0)	22 (81.5)	0.129
Female	10 (37.0)	5 (18.5)	
Histological grade			
Well and moderately	15 (55.6)	17 (63.0)	0.580
Poorly and other	12 (44.4)	10 (37.0)	
Lymphatic invasion			
Absent	22 (81.5)	24 (88.9)	0.440
Present	5 (18.5)	3 (11.1)	
Venous invasion			
Absent	22 (81.5)	22 (81.5)	1.00
Present	5 (18.5)	5 (18.5)	
Lymph node metastasis			
N0	15 (55.6)	22 (81.5)	0.040
N1-2	12 (44.4)	5 (18.5)	
Distant metastasis			
Absent	21 (22.2)	26 (96.3)	0.043
Present	6 (77.8)	1 (3.7)	
AJCC stage			
I, II	13 (48.1)	22 (81.5)	0.010
III, IV	14 (51.9)	5 (18.5)	

analysis showed that *UCA1* expression was an independent prognostic indicator of poor survival in CRC (P=0.023) in addition to the presence of lymphatic invasion (P=0.016) (Table III).

Knockdown of UCA1 suppresses cell proliferation and prevents the G0/G1 progression of CRC cells. To investigate the biological function of *UCA1*, we conducted *UCA1* knockdown assays using RNA interference in CRC cells. Firstly, we confirmed that *UCA1* expression in HCT116 and DLD1 cells transfected with *UCA1*-specific siRNA was significantly lower than that in cells transfected with the negative control siRNA: the expression levels of *UCA1* were reduced by 90% and 80%, as detected by qPCR. Real-time cellular analysis (RTCA) indicated that cell proliferation was reduced in CRC cells when *UCA1* was knocked down. For HCT116 cells, we observed a 42.8% reduction in the cell proliferation rate when *UCA1* was silenced (0.28 ±0.07 for the siRNA-UCA1 group vs. 0.49±0.08 for the siControl group, P=0.028) (Fig. 2A). For DLD1 cells, we observed a 21.9% reduction in the cell proliferation rate when *UCA1* was silenced (0.25±0.03 for the siRNA-UCA1 group vs. 0.32±0.02 for the siControl group, P=0.033) (Fig. 2B). In addition, fluorescence-activated cell sorting (FACS) analysis was conducted to analyze the effect of *UCA1* on cell cycle progression. Knockdown of *UCA1* increased the proportion of cells in G0/G1 phases (HCT116 cell line: 73.9±8.57% for the siRNA-UCA1 group vs. 52.1±10.3% for the siControl group, P=0.0074; DLD1 cell line: 70.1±8.92% for the siRNA-UCA1 group vs. 54.7±10.0% for the siControl group, P=0.029, respectively) (Fig. 2C and D). Collectively, these data suggested that silencing of *UCA1* contributed to proliferation inhibition via cell cycle arrest.

Silencing UCA1 promotes apoptosis, inhibits migration and invasion in CRC cells. Consistent with decreased cell proliferation, there was a significant increase in the apoptosis of *UCA1* knockdown cells relative to that of the negative control cells (HCT116 cell line: 25.7±8.8% for the siRNA-*UCA1* group vs. 15.2±7.0% for the siControl group, P=0.020; DLD1 cell line:

Table III. Univariate and multivariate analysis for overall survival (Cox proportional hazards regression model).

Factors	Univariate analysis RR	Univariate analysis P-value	Multivariate analysis RR	Multivariate analysis P-value
Age (years)	0.650	0.477		
Gender	0.549	0.443		
Histological grade	0.497	0.302		
Lymphatic invasion (absent/present)	13.71	0.001[a]	11.91	0.016[a]
Venous invasion (absent/present)	1.678	0.445		
Lymph node metastasis (N0/N1-2)	4.574	0.015[a]	1.119	0.926
Distant metastasis (absent/present)	25.65	0.001[a]	3.984	0.157
AJCC stage	11.17	0.002[a]	3.62	0.443
UCA1 expression (low/high)	5.068	0.038[a]	3.137	0.023[a]

RR, relative risk; [a]P<0.05.

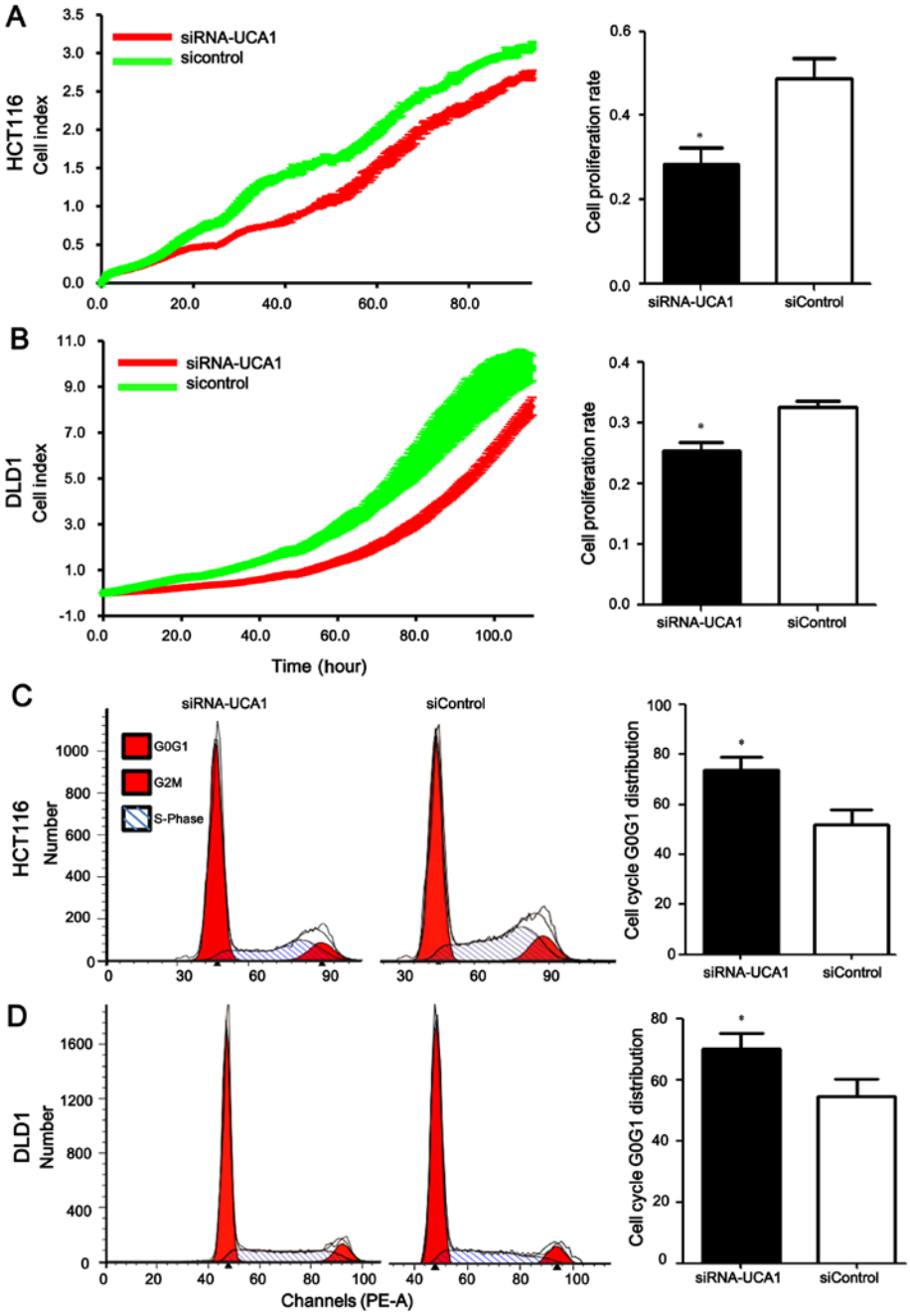

Figure 2. Effects of *UCA1* silencing on proliferation and cell cycle of CRC cells. (A and B) The effect of *UCA1* silencing on cell proliferation was evaluated by RTCA assay in HCT116 and DLD1 cells, respectively (*P<0.05, n=3). (C and D) The cell cycle progression analysis showed *UCA1* silencing promoted the G0/G1 growth arrest of HCT116 and DLD1 cells using FACS analysis, respectively (*P<0.05, n=3).

28.9±3.0% for the siRNA-UCA1 group vs. 21.7±3.3% for the siControl group, P=0.009, respectively) (Fig. 3A). Therefore, the data suggested that *UCA1* could inhibit apoptosis of CRC cells. We further analyzed whether *UCA1* knockdown affected cell migration and invasion of CRC cells using Transwell migration assays. The results indicated that *UCA1*-silenced HCT116 and DLD1 cells showed less potential of migration and invasion ability compared with the negative control cell lines (all P<0.01) (Fig. 3B and C). These data suggested that *UCA1* induced migration and invasion in CRC cells.

Ets-2 is critical for UCA1 promoter activity. To obtain insight into the potential regulators of *UCA1*, we analyzed the core promoter of *UCA1* (from nucleotides -500 to 200) using several bioinformatics software programs and predicted five potential transcription factor binding sites (Ets-2, C/EBPα, c-myb, GATA-1 and CREB) (Fig. 4A and B). To investigate which transcription factor is critical for *UCA1* promoter activity, we replaced the five transcription factor binding sites to mutated sites in pGL3-*UCA1*-700-mut and the mutated sites are shown in Fig. 4C. Compared with the wild-type pGL-3-*UCA1*-700 construct, HCT116 and DLD1 cells yielded a lower promoter activity when transcription factor Ets-2 binding site was mutated, as assessed by luciferase reporter assays (0.677±0.06 for Ets-2 vs. 1 for control, P=0.0084, 0.725±0.08 for Ets-2 vs. 1 for control, P=0.019, respectively) (Fig. 4D). The results

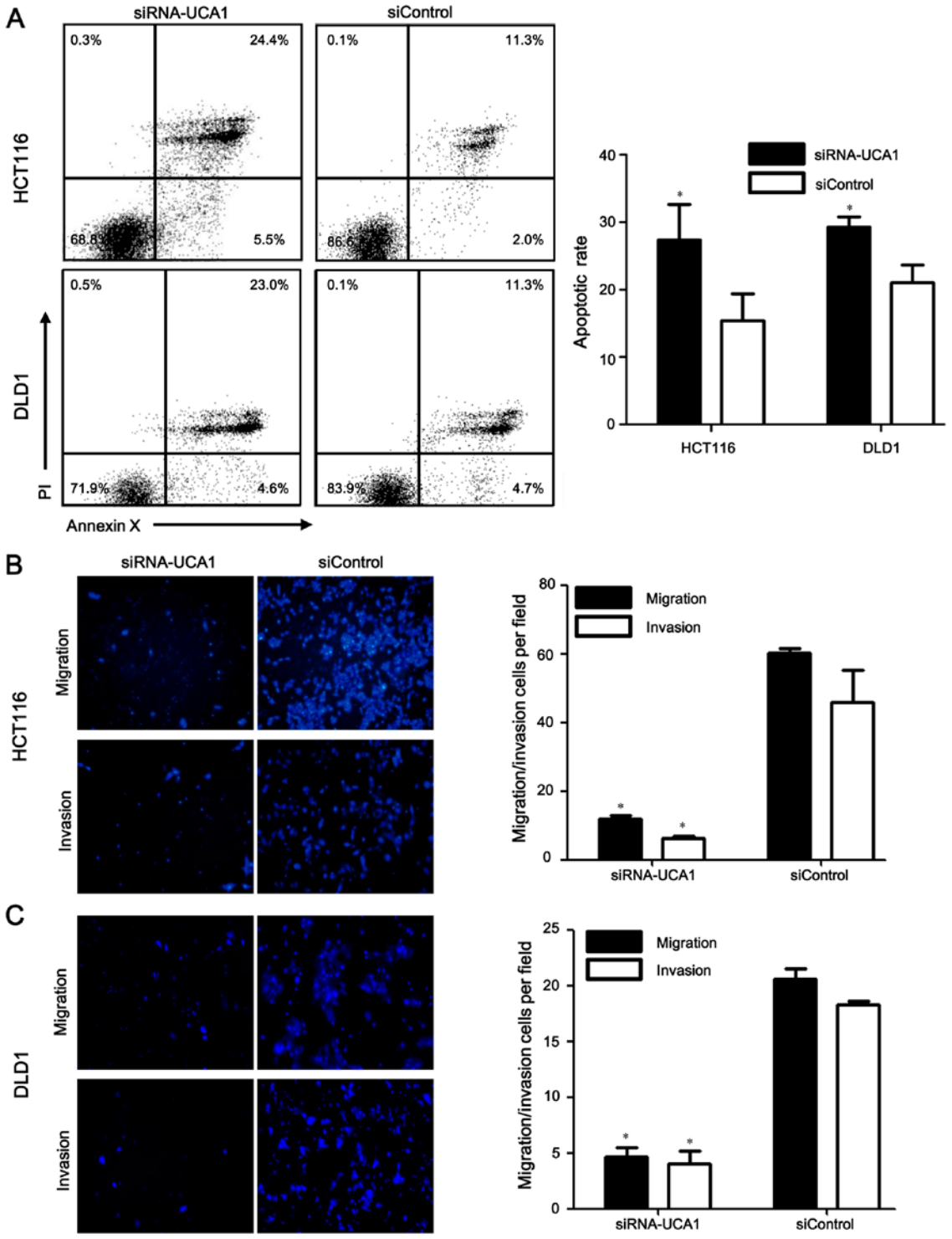

Figure 3. Effects of *UCA1* silencing on the apoptosis, migration and invasion of CRC cells. (A) Silencing of *UCA1* promoted apoptosis of HCT116 and DLD1 cells by FACS analysis following Annexin V and PI staining (*P<0.05, n=3). (B and C) Silencing of *UCA1* decreased the migration and invasion of HCT116 and DLD1 cells (*P<0.05, n=3).

suggested that Ets-2 binding sites play an essential role in the regulation of the *UCA1* promoter activity.

Discussion

CRC is the process whereby benign polyps develop into adenoma and then into tumors; thus, early screening and diagnosis are important to decrease the death rate (21). Numerous genetic and epigenetic alterations, such as DNA methylation and microRNAs, have been studied as potential biomarkers for screening or diagnosis in CRC. However, few such markers can be applied for clinical diagnosis and treatment. Thus, it is essential to screen out new effective biomarkers.

LncRNAs have attracted increased interest from researchers in recent years. A growing number of studies (22-24) suggest that abnormal expressions of lncRNAs are

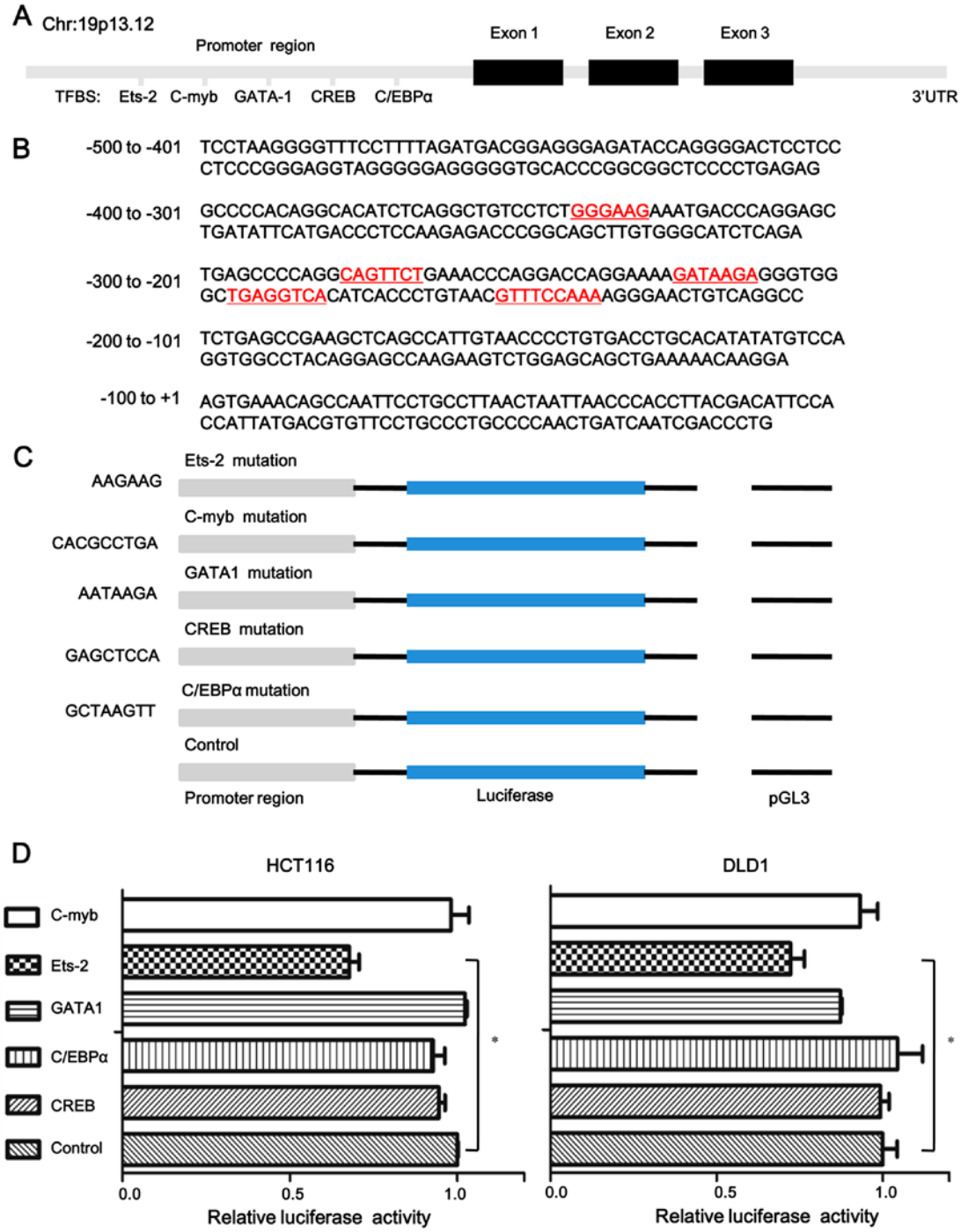

Figure 4. Ets-2 binds with *UCA1* core promoter and contributes to *UCA1* transcriptional activation in CRC cells. (A) *UCA1* genomic structure and the transcription factor binding sites in the *UCA1* core promoter. (B) Sequence of *UCA1* core promoter. The underlining indicates the sequences of binding site of Ets-2, c-myb, GATA1, CREB and C/EBPα, respectively. (C) Schematic representation of the reporter gene constructs containing the 700 bp *UCA1* promoter region. The only differences among these constructs were the mutations of the transcription factor binding sites. (D) Luciferase activity of pGL3-*UCA1*-700-mut constructs in HCT116 and DLD1 cells. pGL3-*UCA1*-700-control construct as positive control vector. Columns, means of three individual experiments; bars, standard deviation (*P<0.05, n=3).

closely related to tumor initiation and progression. Unlike protein-coding mRNAs, which are expressed in multiple tissue types, most lncRNAs show tissue-specific expression patterns. For example, Yang *et al* (25) found that CCAT1 was upregulated in gastric cancer and might be a potential therapeutic marker. Q*i et al* (26) also suggested that low expression of lncRNA loc285194 was related to poor prognosis in CRC. There is no doubt that lncRNAs have significant roles in cancer progression and development. However, only a limited number of lncRNAs have been investigated in detail (27-29) and the functional roles of lncRNAs in CRC are not yet well elucidated.

In the present study, we observed that UCA1 was highly expressed in CRC tissues, suggesting a positive role for *UCA1* in CRC tumourigenesis. In addition, the expression of *UCA1* was associated with lymph mode metastasis (P=0.04), distant

metastasis (P=0.043) and tumor stage (P=0.01). In the survival analysis, high *UCA1* expression levels were related with poor prognosis, indicating that *UCA1* could be a valuable prognostic biomarker. In fact, *UCA1* has been extensively studied in the context of cancer (30-32), especially in bladder cancer (33). Fang *et al* (34) have reported that a significantly elevated expression of *UCA1* in tongue squamous cell carcinomas was found and there was higher expression of *UCA1* in lymph node metastases than in paired primary tumors. Previous studies indicated that other lncRNAs were also considered as molecular biomarkers in CRC. *CCAT1* (35), an upregulated lncRNA in CRC, was explored for early screening and detection. Elevated levels of lincRNA-p21 were significantly associated with CRC disease state. In this regard, *UCA1* could be used as a potential prognostic indicator in CRC.

We identified the biological functions of *UCA1* in CRC cells. Proliferative activity and the ability to metastasis were significantly suppressed *in vitro* after silencing of *UCA1*. Besides, knockdown of *UCA1* induced G0/G1 phase arrest and promoted apoptosis in CRC cells. Similar to these findings, Fan *et al* (20) reported that ectopic UCA1 expression enhances the tumorigenic potential and increases invasion. Some of our results were similar to those of Han *et al* (36), who also observed that elevated *UCA1* expression in CRC could influence cancer cell proliferation and apoptosis. However, our results differ in terms of the metastatic phenotype. Our results showed that high expression of *UCA1* could significantly enhance migration and invasion of CRC cell lines, whereas those of Han *et al* did not. The contradictory results may be due in part to the different cells used. The results suggest that *UCA1* acts as an oncogene in CRC.

Recent studies put forward that the transcription factors regulate lncRNAs via binding with the promoters of lncRNAs, which has been proved to play a pivotal role in tumor progression (37-39). In the present study, we found that Ets-2 could bind to the *UCA1* core promoter and stimulated *UCA1* transcriptional activation in CRC cells. Similar to these findings, Wu *et al* (40) have also demonstrated that Ets-2 bound to the *UCA1* promoter region and regulated cell apoptosis via Akt pathway in bladder cancer. Our data support that Ets-2 takes part in CRC development, but the exact regulatory mechanisms of *UCA1* by Ets-2 in CRC require further investigation.

Some studies have reported that *UCA1* may act as a switch to regulate gene expression in different cell signal pathways in cancer. Yang *et al* (38) observed that *UCA1* regulated the cell cycle through CREB via the PI3K-AKT pathway in bladder carcinoma. In another study, *UCA1* was observed to regulate bladder cancer cell glucose metabolism through the cascade of mTOR-STAT3/miR143-HK2 (41). These results highlighted the effect of *UCA1* on signal transduction pathways. Besides, the report that *UCA1* could be regulated by hsa-miR-1 in bladder cancer showed that lncRNAs may act as a novel set of targets for microRNA (42). Other regulatory factors such as histone modification and DNA methylation that influence *UCA1* expression in CRC need to be further studied.

Our results showed that *UCA1* expression was significantly increased in CRC tissues and cell lines. Elevated levels of *UCA1* were statistically correlated with lymph mode metastasis, distant metastasis and tumor stage, and predicted poor prognosis in CRC. *In vitro*, we demonstrated that *UCA1* was associated with tumor migration, invasion and proliferation of CRC cells. These findings provide important insight into exploring new biomarkers for the diagnosis and therapy of CRC. The results indicate that *UCA1* may be a promising target for future therapy of CRC.

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