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Augmented expression levels of lncRNAs *ecCEBPA* and *UCA1* in gastric cancer tissues and their clinical significance

Mojdeh Nasrollahzadeh-Khakiani [1], Modjtaba Emadi-Baygi [2, 3], Parvaneh Nikpour [1, 4*]

[1] Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2] Department of Genetics, School of Basic Sciences, Shahrekord University, Shahrekord, Iran
[3] Research Institute of Biotechnology, School of Basic Sciences, Shahrekord University, Shahrekord, Iran
[4] Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non-communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

Objective(s): As the second cause of cancer death, gastric cancer (GC) is one of the eminent dilemmas all over the world, therefore investigating the molecular mechanisms involved in this cancer is pivotal. Unrestricted proliferation is one of the characteristics of cancerous cells, which is due to deficiency in cell regulatory systems. Long non-coding RNAs (lncRNAs) have emerged as critical regulators of the epigenome. lncRNA *extra coding CEBPA* (*ecCEBPA*) is involved in DNA methylation. This lncRNA reduces *CEBPA* promoter methylation by interacting with DNA methyltransferase 1. lncRNA *UCA1* (urothelial carcinoma-associated 1) elevates cell proliferation through the PI3K/Akt signaling pathway which has a critical role in cell growth and apoptosis. The aim of this study was to examine the expression of *ecCEBPA* and *UCA1* genes in GC tissues as well as their clinical significance.

Materials and Methods: Total RNA extraction, cDNA synthesis, and quantitative real-time PCR were performed for cells and 80 paired GC tissues. Furthermore, clinical relevance of *UCA1* expression was investigated in TCGA cohort data.

Results: Our results showed *ecCEBPA* and *UCA1* over-expression in GC tissues. Furthermore, lncRNAs associations with clinicopathological features were demonstrated both in the current and TCGA cohort. Kaplan-Meier analysis indicated that patients with higher *UCA1* expression had a worse overall survival in the case of pancreatic and lung adenocarcinomas but not other solid cancer types including GC.

Conclusion: These data demonstrate *UCA1* and *ecCEBPA* involvement in GC and suggest that these lncRNAs might be useful as diagnostic/ prognostic biomarkers in cancer.

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Introduction

Gastric cancer (GC) terminates the lives of a plenty of people every year and is still the second most prevalent cause of cancer deaths worldwide. Stomach cancer progression is a multistep process including alteration in various genes. In spite of developments in diagnostic methods, GC is usually recognized late, therefore examining molecular biomarkers and mechanisms is pivotal for early GC detection (1). Progression in transcriptome analysis has revealed that about 70% of human genome is transcribed into RNAs that do not act as templates for proteins. These RNAs that are referred to as non-coding RNAs (ncRNAs) are classified into different subsets based on their length. lncRNAs are classified as ncRNAs with at least 200 nucleotides length that lack an open reading frame of significant length. lncRNAs have emerged as regulatory players in abundant biological functions such as gene regulation, epigenetic regulation, transcription, mRNA splicing, and translation (2).

Located on human 19p13.12, Urothelial carcinoma associated 1 (*UCA1*) is a lncRNA with three exons (3). *UCA1* over-expression has been reported in different cancer types (4-9). It has been shown that *UCA1* affects p27 expression by interacting with heterogeneous nuclear ribonucleoprotein I and inhibits the p27 protein resulting in elevation of tumor growth by increasing proliferation in breast cancer (10). CREB (cAMP responsive element binding protein) transcription factor, which is involved in augmenting cancer progression, is activated by *UCA1* through AKT kinase in the PI3K/AKT pathway (11). Furthermore, it has been demonstrated that *UCA1* over-expression stimulates cell cycle progression and tumor growth in colorectal cancer cells (12). One of the studied molecules that has a binding site on *UCA1* promoter is CCAAT/enhancer-binding protein α (C/EBPα) that increases *UCA1* expression, which in turn induces cell viability and reduces cell apoptosis in bladder cancer (13). *UCA1* up regulation leads to cyclin D1 over expression which

**Corresponding author:* Parvaneh Nikpour. Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. Tel: +98-31-37929143; Fax: +98-31-37753480; email: pnikpour@med.mui.ac.ir

promotes cell cycle progression in GC (14). The interaction between *UCA1* and miR-182 has been reported in glioma tissues and cell lines (15). Moreover, a recent study on GC has shown that *UCA1* is negatively associated miR-27b expression (16).

Extra coding CEBPA (*ecCEBPA*) is a non-polyadenylated lncRNA that is located on the upstream region of the *CEBPA* locus on chromosome 19. *ecCEBPA* is involved in DNA methylation. This lncRNA interacts with DNA methyltransferase 1 and diminishes *CEBPA* promoter methylation leading to CEBPA up-regulation. *ecCEBPA* expression has been shown in Hl-60 and U937 cell lines (17).

According to these findings, we designed a study to evaluate *UCA1* and *ecCEBPA* expression patterns in GC specimens as well as their correlation with clinicopathological parameters. Furthermore, we analyzed *UCA1* gene expression and clinicopathological characteristics data of this lncRNA in GC from the Cancer Genome Atlas (TCGA) database.

Materials and Methods

Tumoral and non-tumoral tissues

The gastric tissues were acquired from Iran Tumoral Bank (Tehran, Iran). Biological materials were provided by Iran National Tumor Bank which is funded by Cancer Institute of Tehran University for Cancer Research (18-20). All tissue specimens were examined for gene expressions which consisted of 40 tumoral and 40 non-tumoral paired tissue samples. The scheme of this experiment was approved by Ethics Committee of Isfahan University of Medical Sciences. Additionally, written informed consents were obtained from all patients, preceding their participation, by Iran Tumoral Bank.

RNA sequence data sets and differential expression

The data set from an independent cohort in the TCGA database (http://cancergenome.nih.gov) was utilized for the evaluation of *UCA1* lncRNA gene expression and its clinicopathological relevance. The lncRNA reads per kilobases per million reads (RPKM) expression value in TCGA database was downloaded through The Atlas of Noncoding RNAs in Cancer (TANRIC), which contains 285 GC and 33 non-tumoral tissues (21, 22). Clinical information about these 318 patients was also downloaded from TCGA database. Furthermore, TCGA overall survival (OS) data were retrieved from OncoLnc database (23).

Cell culture

The human cell lines HEK-293 (human embryonic kidney 293 cells), HUVECs (human umbilical vein endothelial cells), SKBR3 (human breast cancer cells), A542 (human pulmonary carcinoma cells), MCF7 (human breast cancer cells), and NT2 (human embryonic carcinoma cell line, NTERA2) were cultured in high glucose DMEM (Gibco Life Technologies, Karlsruhe, Germany), supplemented with 15% fetal calf serum, 100 U/ml penicillin, and 10 µg/ml streptomycin. The human cancer cell line HepG2 (hepatocellular carcinoma cell line) was cultivated in RPMI-1640 (Gibco Life Technologies, Karlsruhe, Germany), enriched with 10% fetal calf serum, 100 U/ml penicillin, and 10 µg/ml streptomycin.

RNA extraction, DNase I treatment and cDNA synthesis

RNA extraction for cells and tissues was performed by using TRIzol® reagent (Invitrogen, Carlsbad, California, United states) according to the provided instructions by the manufacturer. RNA integrity was assessed using 1% agarose gel electrophoresis and RNA concentration was checked by the Nanodrop instrument (NanolytiK, Duesseldorf, Germany). DNase I treatment was performed using DNase set (Fermentas, Vilnius, Lithuania) in order to prepare DNA-free RNA prior to RT-PCR. cDNA was synthesized by using PrimeScript™RT reagent Kit (TaKaRa, Kusatsu, Shiga, Japan).

Quantitative real-time PCR

The relative expression of lncRNA *UCA1* and *ecCEBPA* were measured by quantitative real-time RT-PCR with specific primers designed using the GeneRunner software package, version 4.0 (Table 1). Primers for amplification of the GUSB (β-Glucuronidase) gene (as an internal control) were taken from another study (24). PCR was performed using RealQ Plus 2x Master Mix Green (high Rox) (Ampliqon, Odense M, Denmark) on an Applied Biosystems StepOnePlus™ instrument. The PCR cycling conditions consisted of a first denaturation step at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 sec, annealing at 61°C for lncRNA *UCA1* and *ecCEBPA,* and at 60 °C for *GUSB* genes and then extension for 15 sec at 72 °C. Additionally, the specificity of PCR amplicons was verified by Sanger sequencing using Applied Biosystems 3730XL sequencer (Macrogen, Seoul, South Korea).

Statistical analysis

Relative gene expression was calculated using the ΔC_t method (Ct of lncRNA minus Ct of housekeeping gene). All experiments were replicated at least 2-3 times and acquired data are represented as mean± standard error of mean (SEM). Kolmogorov-Smirnov test was implemented in order to find out the normal distribution of samples. The results were analyzed using Student's t-test, ANOVA (analysis of variance), and chi-square. Kaplan-Meier and Cox regression analyses were

Table 1. Primer sequences for amplification of *ecCEBPA, UCA1,* and *GUSB**

Primers	Sequence	Amplicon size
hecCEBPA-F1	TTGGCGAGGCTTCTTATCTG	133 bp
hecCEBPA-R1	GCTGCAGCTGTAGGTGATTTG	
hUCA1-F1	ATCGGATCTCCTCGGCTTAG	145 bp
hUCA1-R1	TGATCGTCCAGCTAGGGTGTC	
hGUSB-F1	CACGACACCCACCACCTACATC	121 bp
hGUSB-R1	GACGCACTTCCAACTTGAACAG	

* Primer sequences were derived from this reference (24)

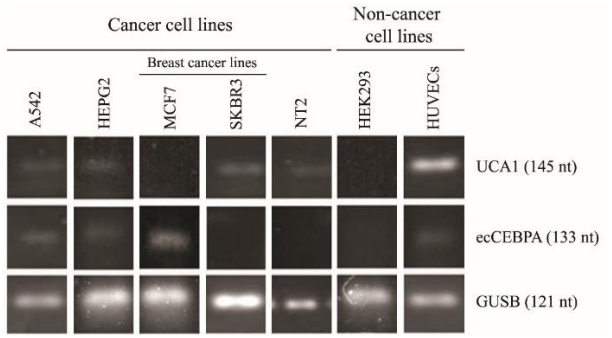


Figure 1. Expression of *ecCEBPA, UCA1,* and *GUSB* transcripts in various cell lines. Electrophoresis results of *ecCEBPA*, *UCA1,* and *GUSB* PCR products on the agarose gel are presented in the figure

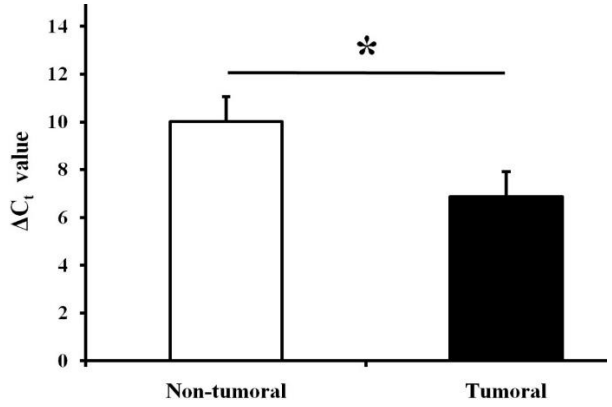


Figure 2. Relative expression of *UCA1* in tumoral and non-tumoral gastric tissue samples (n=40). A lower ΔC_t value shows higher expression levels. Values shown represent the mean±SEM. Asterisk represents a statistically significant difference ($P \leq 0.05$) Error bars stand for standard error of mean (SEM)

utilized to assess the association between lncRNA *UCA1* and overall survival. The SPSS software, version 16.0 (SPSS Inc., Chicago IL), was used for statistical analysis. Figures were made by GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA). A *P*-value of less than 0.05 was considered a significant difference.

Results

Expression profile of UCA1 and ecCEBPA in various cell lines

Optimization of *UCA1* was performed on the HepG2 cell line, as previously reported in a study (9), *UCA1* is expressed in these cells. Agarose gel electrophoresis showed a specific band with the expected size. Additionally, real-time RT-PCR reaction for the examined genes showed a unique melting curve without primer dimers. A few PCR products were further sequenced to confirm lncRNAs specific amplification (data not shown). *UCA1* expression was detected in HUVECs, SKBR3, A542, NT2, and HepG2 cell lines whereas it was not observed in HEK-293 and MCF7 cells. Moreover, *ecCEBPA* expression evaluation on the mentioned cell lines revealed their expression in

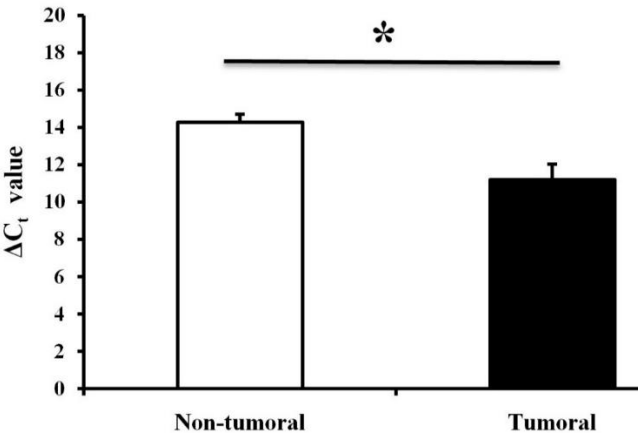


Figure 3. Relative expression of *ecCEBPA* in tumoral and non-tumoral gastric tissue samples (n=40). A lower ΔC_t value shows higher expression levels. Values shown represent the mean ± SEM Asterisk represents a statistically significant difference ($P \leq 0.05$) Error bars stand for standard error of mean (SEM)

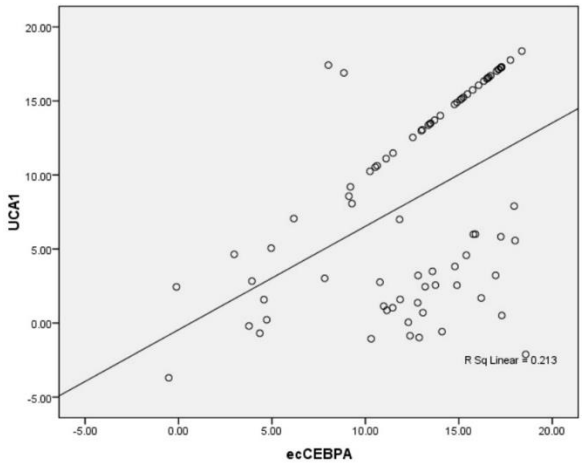


Figure 4. Correlation analysis of lncRNAs *UCA1* and *ecCEBPA* gene expression levels (*P*-value=0.000, r=0.46)

HUVECS, A542, HepG2, HEK-293, and MCF7 cells but not in SKBR3 and NT2 cultured cells (Figure 1).

Augmented expression of lncRNA UCA1 and ecCEBPA in gastric cancer tissues

The expression levels of *UCA1* and *ecCEBPA* were measured by quantitative real-time PCR in 80 pairs of GC and their adjacent non-tumoral tissues. Specific primers were used for both lncRNAs and *GUSB* (as a reference gene). The ΔC_t method was applied to examine the relative expression levels of *UCA1* and *ecCEBPA*. As presented in Figure 2, *UCA1* relative expression showed an increase in tumoral tissues (*P*-value=0.036) compared with the adjacent non-tumoral ones (6.867±1.03 versus 10.00±1.05, respectively). As shown in Figure 3, the relative expression status of *ecCEBPA* was significantly elevated in tumoral tissues (*P*-value=0.001) compared with the adjacent non-tumoral ones (11.187±0.82 versus 14.254±0.44, respec-

Table 2. Relationship between *UCA1* mean expression levels (ΔC_t^*) and clinicopathological characteristics of tumoral gastric specimens

Characteristics	Number (#40)	Mean ± SEM	*P*-value
Sex			0.29
Male	24	7.50±1.4	
Female	16	5.90±1.52	
Age (years)			0.24
≥70	18	7.92±1.61	
<70	22	6.0±1.33	
Depth of invasion			0.31
T2	3	9.19±3.81	
T3-4	37	6.67±1.08	
N classification			0.30
NX-N0	7	5.11±2.37	
N1	20	7.94±1.56	
N2-3	13	6.39±1.74	
M classification			0.050**
MX	8	4.46±2.23	
M0	24	8.47±1.27	
M1	8	4.44±2.41	
TNM stages			0.21
I-II	22	7.94±1.43	
III	11	9.2±1.92	
IV	7	4.44±2.41	
Perineural invasion	10	6.75±2.53	0.34
Negative	30	6.9±1.11	
Positive			
Lymphatic invasion	14	5.36±1.83	0.12
Negative	26	7.67±1.24	
Positive			
Tumor size			0.30
≥5	31	6.58±1.18	
<5	9	7.85±2.18	
Tumor grades			0.01**
I	12	5.11±2.0	
II	9	4.32±2.05	
III	19	9.44±1.32	
Tumor types			0.04**
Diffuse	19	8.35±1.44	
Intestinal	21	5.519±1.43	

* A lower ΔC_t value indicates a higher expression level
** Statistically significant

tively). Note that a lower ΔCt indicates a higher relative expression.

Association of UCA1 and ecCEBPA with various clinicopathological parameters

As shown in Tables 2 and 3, data analysis showed no meaningful correlation between *UCA1* and *ecCEBPA* expression levels with clinicopathological characterristics, except for significant associations between the expression level of lncRNA *UCA1* and tumor grades (*P*-value=0.01), tumor type (*P*-value=0.04), and M classification (*P*-value=0.05). Furthermore, GC samples were classified into high (#20) and low (#20) expression groups based on median value of lncRNAs expression levels in tumoral specimens. As presented in Tables 4 and 5, results demonstrate no correlation between *UCA1* and *ecCEBPA* expression levels and any of the clinicopathological parameters. Furthermore, a Pearson correlation analysis between *UCA1* and *ecCEBPA* relative expression levels in GC tissues revealed a moderate positive correlation (*P*-value =0.000, r=0.46) (Figure 4).

Table 3. Relationship between *ecCEBPA* mean expression levels (ΔC_t^*) and clinicopathological characteristics of tumoral gastric specimens

Characteristics	Number (#40)	Mean± SEM	*P*-value
Sex			0.45
Male	24	11.22±1.33	
Female	16	11.13±1.06	
Age (years)			0.15
≥70	18	12.25±1.09	
<70	22	10.31±1.19	
Depth of invasion			0.25
T2	3	10.18±2.82	
T3-4	37	11.26±0.87	
N classification			0.20
NX-N0	7	9.16±2.02	
N1	20	11.83±1.20	
N2-3	13	11.35±1.39	
M classification			0.24
MX	8	10.50±1.90	
M0	24	10.89±1.04	
M1	8	12.76±2.02	
TNM stages			0.21
I-II	22	11.01±1.17	
III	11	10.36±1.42	
IV	7	12.76±2.02	
Perineural invasion			0.20
Negative	10	14.57±0.63	
Positive	30	10.89±0.93	
Lymphatic invasion			0.23
Negative	14	10.32±1.57	
Positive	26	11.65±0.95	
Tumor size			0.30
≥5	31	10.84±1.95	
<5	9	12.36±4.12	
Tumor grades			0.35
I	12	11.11±1.67	
II	9	9.66±1.97	
III	19	12.07±0.99	
Tumor types			0.24
Diffuse	19	10.97±1.10	
Intestinal	21	11.37±1.23	

* A lower ΔC_t value indicates a higher expression level

Association of UCA1 expression level with clinical data in the TCGA stomach cancer cohort

The authors explored the expression levels of *UCA1* lncRNA in a TCGA stomach cancer (STAD) cohort. We found that *UCA1* was significantly overexpressed in gastric tumoral tissues compared with normal tissues (*P*<0.0001, Figure 5). Further analysis based on *UCA1* mean expression data showed that there is a significant correlation between *UCA1* gene expression levels and M classification (*P*=0.01) and various Lauren`s classes (*P*=0.0009) (Table 6). Patients were then divided into low and high *UCA1* expression groups according to the median value. The results demonstrated that there were significant associations between *UCA1* gene expression level and M classification (*P*=0.04), TNM stages (*P*=0.01), tumor grades (*P*=0.0002), and Lauren`s classes (*P*=0.0002) (Table 7).

High expression levels of UCA1 were correlated with unfavorable survival in patients with pancreatic and lung adenocarcinomas

We furthermore examined whether *UCA1* expression level correlates with patient overall survival time across

Table 4. Relationship between *UCA1* expression levels (as divided into two groups based on the median of ΔC_t) and clinicopathological characteristics of tumoral gastric specimens

Characteristics	Number (#40)	lncRNA *UCA1* expression		*P*-value
		Low (higher ΔC_t than median) (#20)	High (smaller ΔC_t than median) (#20)	
Sex				0.37
Male	24	13	11	
Female	16	7	9	
Age (years)				0.50
≥70	18	9	9	
<70	22	11	11	
Depth of invasion				0.50
T2	3	2	1	
T3-4	37	18	19	
N classification				0.21
NX-N0	7	3	4	
N1	20	12	8	
N2-3	13	5	8	
M classification				0.06
MX	8	2	6	
M0	24	15	9	
M1	8	3	5	
TNM stages				0.17
I-II	22	13	9	
III	11	5	6	
IV	7	2	5	
Perineural invasion				0.07
Negative	10	4	6	
Positive	30	16	14	
Lymphatic invasion				0.16
Negative	14	5	9	
Positive	26	15	11	
Tumor size				0.50
≥5	31	15	16	
<5	9	5	4	
Tumor grades				0.13
I	12	5	7	
II	9	3	6	
III	19	12	7	
Tumor types				0.26
Diffuse	19	11	8	
Intestinal	21	9	12	

Table 5. Relationship between *ecCEBPA* expression levels (as divided into two groups based on the median of ΔC_t) and clinicopathological characteristics of tumoral gastric specimens

Characteristics	Number (#40)	lncRNA *ecCEBPA* expression		*P*-value
		Low (higher ΔC_t than median) (#20)	High (smaller ΔC_t than median) (#20)	
Sex				0.36
Male	24	13	11	
Female	16	7	9	
Age (years)				0.37
≥70	18	10	8	
<70	22	10	12	
Depth of invasion				0.50
T2	3	2	1	
T3-4	37	18	19	
N classification				0.22
NX-N0	7	2	5	
N1	18	10	8	
N2-3	15	8	7	
M classification				0.12
Mx	8	3	5	
M0	24	11	13	
M1	8	6	2	

Table 5. Continued

TNM stages				0.13
I-II	21	9	12	
III	11	5	6	
IV	8	6	2	
Perineural invasion				0.13
Negative	10	7	3	
Positive	30	13	17	
Lymphatic invasion				0.50
Negative	14	7	3	
Positive	26	13	17	
Tumor size				0.135
≥5	30	13	17	
<5	10	7	3	
Tumor grades				0.46
I	12	6	6	
II	9	4	5	
III	19	10	9	
Tumor types				0.50
Diffuse	19	9	10	
Intestinal	21	11	10	

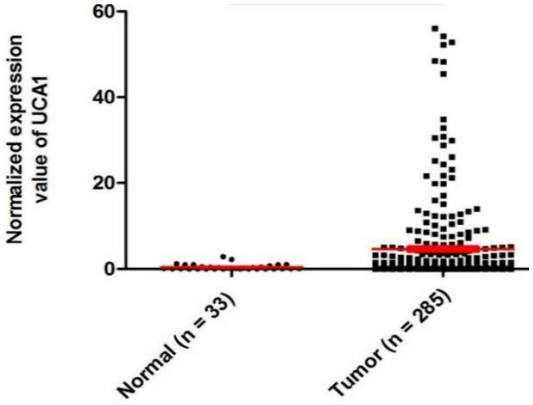
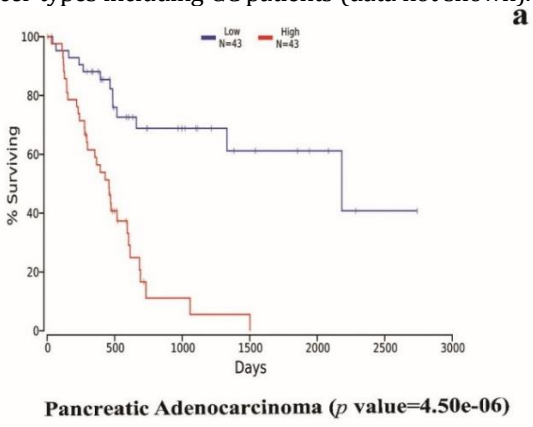


Figure 5. Relative expression of *UCA1* in tumoral and non-tumoral gastric tissue samples in the TCGA database. *UCA1* was found to be highly overexpressed in tumoral tissues compared with normal tissues in the TCGA RNA-seq data (P<0.0001)

multiple solid cancer types (based on *P*-values from the univariate Cox proportional hazards model and the log-rank test and visualization through a Kaplan-Meier plot). As shown in Figure 6, elevated *UCA1* expression was contributed to a significant poorer survival in patients with pancreatic (P=4.5×10^{-6}) and lung adenocarcinomas (P=7.3×10^{-3}) but not with survival time of other solid cancer types including GC patients (data not shown).



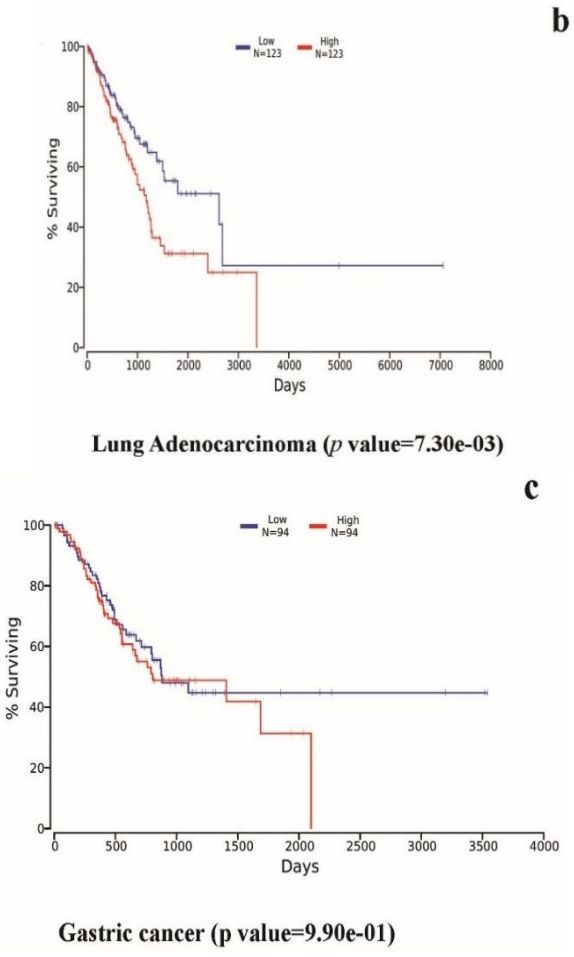


Figure 6. Kaplan-Meier curves for overall survival of TCGA cohort patients with pancreatic, lung, and gastric adenocarcinomas categorized according to *UCA1* expression: significantly poorer overall survival was observed in patients with high *UCA1* expression than in those with low *UCA1* expression (a and b). There was no significant association between *UCA1* expression and overall survival of TCGA cohort gastric cancer patients (c)

Table 6. Relationship between *UCA1* mean expression levels and clinicopathological characteristics of tumoral gastric specimens in the TCGA cohort

Characteristics	Numbers (%)	Mean±SEM	*P*-value
Sex			0.20
Male	174 (61.05)	5.25±0.79	
Female	111 (38.95)	3.64±.079	
Age (years)			0.07
≥70	113 (39.65)	5.46±1.01	
<70	167 (58.60)	3.98±.070	
NA*	5 (1.75)		
Depth of invasion			0.20
T1	13 (4.56)	2.018±0.66	
T2	72 (25.26)	4.90±1.18	
T3	113 (39.65)	5.84±1.05	
T4	78 (27.37)	2.82±0.82	
TX	9 (3.16)	6.52±4.23	
N classification			0.07
N0	94 (32.98)	4.27±0.92	
N1	78 (27.37)	4.53±0.89	
N2	47 (16.49)	6.63±1.88	
N3	53 (18.60)	3.86±1.45	
NX	13 (4.56)	3.83±2.86	
M classification			0.01**
M0	253 (88.77)	4.06±0.50	
M1	18 (6.32)	6.30±4.05	
MX	14 (4.91)	12.68±4.99	
TNM stage			0.40
I	40 (14.03)	4.45±1.44	
II	99 (34.74)	4.20±0.85	
III	101 (35.44)	4.86±0.99	
IV	25 (8.77)	5.69±2.93	
NA	20 (7.02)		
Tumor grades			0.11
G1-GX	12 (4.21)	2.75±1.28	
G2	91 (31.93)	4.20±1.69	
G3	182 (63.86)	3.49±0.97	
Tumor types			0.0009**
Diffuse	51 (17.89)	3.81±1.54	
Intestinal	95 (33.33)	4.53±0.90	
Mixed	136 (47.72)	5.06±0.85	
NA	3 (1.05)		

* Not available
** Statistically significant

Table 7. Relationship between *UCA1* expression levels and clinicopathological characteristics of tumoral gastric specimens of the TCGA cohort based on median gene expression level

Characteristics	Numbers (#285)	*UCA1* expression		*P*-value
		A higher expression than median (#142)	A lower expression than median (#143)	
Sex				0.35
Male	174	91	83	
Female	111	51	60	
Age (years)				0.15
≥70	113	62	51	
<70	167	76	91	
NA*	5	4	1	
Depth of invasion				0.29
T1	13	8	5	
T2	72	41	31	
T3	113	57	56	
T4	78	33	45	
TX	9	3	6	
N classification				0.22
N0	94	51	43	
N1	78	39	38	
N2	47	27	20	
N3	53	20	33	
NX	13	5	8	

Table 7. Continued

M classification				0.04**
M0	253	127	126	
M1	18	5	13	
MX	14	10	4	
TNM stage				0.01**
I	40	24	16	
II	99	52	27	
III	101	49	52	
IV	25	8	17	
NA	20	9	11	
Tumor grades				0.0002**
G1-GX	12	7	5	
G2	91	61	30	
G3	182	74	108	
Tumor types				0.0002**
Diffuse	51	13	38	
Intestinal	95	58	37	
Mixed	136	70	66	
NA	3	1	2	

* Not available
** Statistically significant

Discussion

In the present study, we evaluated and quantified the expression level of the *ecCEBPA* gene in tumoral and non-tumoral gastric tissues as well as various cultured cell lines by using quantitative real-time PCR. To our knowledge, the expression profiling of *ecCEBPA* has not been previously studied in GC. Our results indicate that *ecCEBPA* expression level was elevated in tumoral tissues compared to the non-tumoral samples. We observed no associations between this lncRNA expression pattern and any of the clinicopathological features. According to the only published study on *ecCEBPA* conducted by Di Ruscio *et al.* (17), *ecCEBPA* is a non-polyadenylated lncRNA which is transcribed from the *CEBPA* locus and is enriched in nuclear fraction. It has been stated that *ecCEBPA* associates physically with DNMT1 which leads in blocking DNA methylation in *CEBPA* promoter and maintains the mRNA expression on from this locus. According to that study, *CEBPA* promoter DNA demethylation, which is mediated by *ecCEBPA,* is almost selective for *CEBPA* locus. Furthermore, they have reported *ecCEBPA* expression in Hl-60 and U937 cell lines by using strand specific reverse transcriptase PCR and northern blot analysis.

Furthermore, our study demonstrated *UCA1* over-expression in tumoral gastric tissues in comparison to non-tumoral ones. Additionally, in the present study, we analyzed stomach cancer datasets based on the TCGA platform and showed that the expression of *UCA1* was significantly higher in GC tissues. Clinically, increased expression of *UCA1* was a predictor of OS in pancreatic and lung adenocarcinoma patients in the TCGA corhort. We could further detect a significant association between *UCA1* expression levels and tumor grades, tumor type, and M classification. *UCA1* involvement has been shown in many cancer types. It has been reported that this lncRNA accelerates cell cycle progression and proliferation and also suppresses apoptosis in colorectal cancer cells (12). *UCA1* increases tumor growth and metastasis by raising cell proliferation through repressing p27 in breast cancer tissues (10). According to a recent study in GC cells, *UCA1* promotes epithelial-mesenchymal transition (EMT), an essential early step in tumor metastasis (25). Our data on association between *UCA1* expression levels and M classification, as an indicator of distant metastasis is in agreement with the role of *UCA1* in EMT and metastasis regulation (25).

Researchers (26) have investigated *UCA1* expression pattern in tumoral and non-tumoral gastric tissues (5 pairs) and also in plasma samples of patients with GC and in their pair-matched plasma (20 pairs) by using RT-PCR. According to their study, *UCA1* is up-regulated in tumoral gastric tissues and plasma with a positive correlation between *UCA1* expression in cancerous gastric tissues and plasma. Associations between *UCA1* expression level and lymph node metastasis and staging have also been shown in that study. In another study by Zheng *et al.* (27), expression profile of *UCA1* was measured in tumoral and non-tumoral gastric tissues and juice. Their results demonstrate over-expression of *UCA1* in tumoral samples; they also showed associations between *UCA1* expression and differentiation, tumor size, invasion depth, and TNM stages. Moreover, it was indicated that patients with a high expression level of *UCA1* are likely to have shorter overall and disease-free survival than patients with lower expression. Overall, our study is consistent with the mentioned studies whereas we could show a correlation between *UCA1* expression level and tumor grades, types, stages, and M classification. Up-regulation of *UCA1* has been previously documented in several other cancer types including bladder carcinoma (3), non-small cell lung cancer (4), tongue

squamous cell carcinoma (5), esophageal squamous cell carcinoma (6), ovarian cancer (7), melanoma (8), and hepatocellular carcinoma (9). According to the present information, *UCA1* can serve as an oncogenic lncRNA in a variety of cancers, which makes it a competent therapeutic target.

Another interesting finding of the current study was a moderate positive correlation between *UCA1* and *ecCEBPA* expression levels, which is consistent with the data and hypotheses of Di *Ruscio et al.* (17) and Xue *et al* (13). Generally, our investigations showed up-regulation of both *ecCEBPA* and *UCA1* in GC. Moreover, the associations between *UCA1* and tumor grades, types, stages, and M classification were significant. The elucidation of the molecular mechanisms modulated by these lncRNAs needs further investigation.

Conclusion

This study demonstrates *UCA1* and *ecCEBPA* up-regulation in GC tissues. Higher expression of *UCA1* was associated with tumor grades, types, stages, and M classification. Furthermore, analyzing data taken from TCGA database for *UCA1* expression, showed over-expression of *UCA1* in GC. Moderate co-expression of lncRNAs, *ecCEBPA,* and *UCA1* were also shown in this research. These data indicate *UCA1* and *ecCEBPA* involvement in GC and suggest that these lncRNAs might be useful as diagnostic/prognostic biomarkers in GC.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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