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

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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. IncRNA extra coding CEBPA (ecCEBPA) is involved in DNA methylation. This IncRNA reduces CEBPA promoter methylation by interacting with DNA methyltransferase 1. IncRNA 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.

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

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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 by Sanger sequencing using ecCEBPA, UCA1, and GUSB (TaKaRa, Kusatsu, Shiga, Japan). 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 PrimeScriptTM 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 ΔCt 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 performed.

| Primers | Sequence | Amplion size |
|---------|----------|--------------|
| hUC1A-F1 | TGGCCGAGGCTTCTATCTG | 133 bp |
| hUC1A-R1 | GCTGACCTGAGCTGATTGG | 145 bp |
| hUCA1-F1 | ATGGATCTCTGCGGTAG | 145 bp |
| hUCA1-R1 | TATGCTGAGCTGAGGTC | 121 bp |
| hGUSB-F1 | CAGGACACACCACAGCTACATC | 121 bp |
| hGUSB-R1 | GAGGCATTCAACCTTAGAACG | 133 bp |

* Primer sequences were derived from this reference (24)
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.

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 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 (ΔCt) and clinicopathological characteristics of tumoral gastric specimens**

| Characteristics         | Number (#40) | Mean ± SEM | P-value |
|-------------------------|--------------|------------|---------|
| Sex                     |              |            |         |
| Male                    | 24           | 7.50±1.4   | 0.29    |
| Female                  | 16           | 5.90±1.52  |         |
| Age (years)             |              |            |         |
| ≥70                     | 18           | 7.92±1.61  |         |
| <70                     | 22           | 6.0±1.33   |         |
| Depth of invasion       |              |            |         |
| T2                      | 3            | 9.19±3.81  |         |
| T3-4                    | 37           | 6.67±1.08  |         |
| N classification        |              |            |         |
| NX-N0                   | 7            | 5.11±2.37  |         |
| N1                      | 20           | 7.94±1.56  |         |
| N2-3                    | 13           | 6.39±1.97  |         |
| M classification        |              |            |         |
| MX                      | 8            | 4.46±2.23  |         |
| MX0                     | 24           | 8.47±1.27  |         |
| MX1                     | 8            | 4.44±2.41  |         |
| TNM stages              |              |            |         |
| I-II                    | 22           | 7.94±1.43  | 0.21    |
| III                     | 11           | 9.2±1.92   |         |
| IV                      | 7            | 4.44±2.41  |         |
| Perineural invasion     |              |            | 0.34    |
| Negative                | 10           | 6.75±2.53  |         |
| Positive                | 30           | 6.9±1.11   |         |
| Lymphatic invasion      |              |            | 0.12    |
| Negative                | 14           | 5.36±1.83  |         |
| Positive                | 26           | 7.67±1.24  |         |
| Tumor size              |              |            | 0.30    |
| ≥5                      | 31           | 6.58±1.18  |         |
| <5                      | 9            | 7.85±2.18  |         |
| Tumor grades            |              |            |         |
| I                       | 12           | 5.1±2.0    |         |
| II                      | 9            | 4.32±2.05  |         |
| III                     | 19           | 9.44±1.32  |         |
| Tumor types             |              |            |         |
| Diffuse                 | 19           | 8.35±1.44  |         |
| Intestinal              | 21           | 5.51±1.43  |         |

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

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

| Characteristics         | Number (#40) | Mean ± SEM | P-value |
|-------------------------|--------------|------------|---------|
| Sex                     |              |            |         |
| Male                    | 24           | 11.22±1.33 | 0.45    |
| Female                  | 16           | 11.13±1.06 |         |
| Age (years)             |              |            |         |
| ≥70                     | 18           | 12.25±1.09 | 0.15    |
| <70                     | 22           | 10.31±1.19 |         |
| Depth of invasion       |              |            |         |
| T2                      | 3            | 10.18±2.82 |         |
| T3-4                    | 37           | 11.26±0.87 |         |
| N classification        |              |            |         |
| NX-N0                   | 7            | 9.16±2.02  |         |
| N1                      | 20           | 11.83±1.20 |         |
| N2-3                    | 13           | 11.35±1.39 |         |
| M classification        |              |            |         |
| MX                      | 8            | 10.50±1.90 | 0.24    |
| MX0                     | 24           | 10.89±1.03 |         |
| MX1                     | 8            | 12.76±2.02 |         |
| TNM stages              |              |            |         |
| I-II                    | 22           | 11.01±1.17 | 0.21    |
| III                     | 11           | 10.36±1.42 |         |
| IV                      | 7            | 12.76±2.02 |         |
| Perineural invasion     |              |            |         |
| Negative                | 10           | 14.57±0.63 | 0.20    |
| Positive                | 30           | 10.89±0.93 |         |
| Lymphatic invasion      |              |            |         |
| Negative                | 14           | 10.32±1.57 | 0.23    |
| Positive                | 26           | 11.65±0.95 |         |
| Tumor size              |              |            |         |
| ≥5                      | 31           | 10.84±1.95 | 0.30    |
| <5                      | 9            | 12.36±4.12 |         |
| Tumor grades            |              |            |         |
| I                       | 12           | 11.11±1.67 | 0.35    |
| II                      | 9            | 9.66±1.97  |         |
| III                     | 19           | 12.07±0.99 |         |
| Tumor types             |              |            |         |
| Diffuse                 | 19           | 10.97±1.10 | 0.24    |
| Intestinal              | 21           | 11.37±1.23 |         |

* A lower ΔCt 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) | IncRNA UCA1 expression | P-value |
|------------------------------|--------------|------------------------|---------|
|                              | Low (higher ΔC_t than median) (#20) | High (smaller ΔC_t than median) (#20) |         |
| Sex                          |              |                        |         |
| Male                         | 24           | 13                     | 11      |
| Female                       | 16           | 7                      | 9       |
| Age (years)                  |              |                        |         |
| ≥70                          | 18           | 9                      | 9       |
| <70                          | 22           | 11                     | 11      |
| Depth of invasion            |              |                        |         |
| T2                           | 3            | 2                      | 1       |
| T3-4                         | 37           | 18                     | 19      |
| N classification             |              |                        |         |
| NX-N0                        | 7            | 3                      | 4       |
| N1                           | 20           | 12                     | 8       |
| N2-3                         | 13           | 5                      | 8       |
| M classification             |              |                        |         |
| MX                           | 8            | 2                      | 6       |
| M0                           | 24           | 15                     | 9       |
| M1                           | 8            | 3                      | 5       |
| TNM stages                   |              |                        |         |
| I-II                         | 22           | 13                     | 9       |
| III                          | 11           | 5                      | 6       |
| IV                           | 7            | 2                      | 5       |
| Perineural invasion          |              |                        |         |
| Negative                     | 10           | 4                      | 6       |
| Positive                     | 30           | 16                     | 14      |
| Lymphatic invasion           |              |                        |         |
| Negative                     | 14           | 5                      | 9       |
| Positive                     | 26           | 15                     | 11      |
| Tumor size                   |              |                        |         |
| ≥5                           | 31           | 15                     | 16      |
| <5                           | 9            | 5                      | 4       |
| Tumor grades                 |              |                        |         |
| I                            | 12           | 5                      | 7       |
| II                           | 9            | 3                      | 6       |
| III                          | 19           | 12                     | 7       |
| Tumor types                  |              |                        |         |
| 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) | IncRNA ecCEBPA expression | P-value |
|------------------------------|--------------|---------------------------|---------|
|                              | Low (higher ΔC_t than median) (#20) | High (smaller ΔC_t than median) (#20) |         |
| Sex                          |              |                          |         |
| Male                         | 24           | 13                      | 11      |
| Female                       | 16           | 7                       | 9       |
| Age (years)                  |              |                          |         |
| ≥70                          | 18           | 10                      | 8       |
| <70                          | 22           | 10                      | 12      |
| Depth of invasion            |              |                          |         |
| T2                           | 3            | 2                       | 1       |
| T3-4                         | 37           | 18                      | 19      |
| N classification             |              |                          |         |
| NX-N0                        | 7            | 2                       | 5       |
| N1                           | 18           | 10                      | 8       |
| N2-3                         | 15           | 8                       | 7       |
| M classification             |              |                          |         |
| Mx                           | 8            | 3                       | 5       |
| M0                           | 24           | 11                      | 13      |
| M1                           | 8            | 6                       | 2       |
Table 5. Continued

| TNM stages |  |  |  |
|-------------|---|---|---|
| I-II        | 21| 9 | 12 |
| III         | 11| 5 | 6  |
| IV          | 8 | 6 | 2  |
| Perineural invasion |  |  |  |
| Negative    | 10| 7 | 3  |
| Positive    | 30| 13| 17 |
| Lymphatic invasion |  |  |  |
| Negative    | 14| 7 | 3  |
| Positive    | 26| 13| 17 |
| Tumor size  |  |  |  |
| ≥5          | 30| 13| 17 |
| <5          | 10| 7 | 3  |
| Tumor grades |  |  |  |
| I           | 12| 6 | 6  |
| II          | 9 | 4 | 5  |
| III         | 19| 10| 9  |
| Tumor types |  |  |  |
| 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                        |             |          |         |
| Male                       | 174 (61.05) | 5.25±0.79| 0.20    |
| Female                     | 111 (38.95) | 3.64±0.79|         |
| Age (years)                |             |          |         |
| ≥70                        | 113 (39.65) | 5.46±1.01| 0.07    |
| <70                        | 167 (58.60) | 3.98±0.70|         |
| NA*                        | 5 (1.75)    |          |         |
| Depth of invasion          |             |          | 0.20    |
| T1                         | 13 (4.56)   | 2.01±0.66|         |
| T2                         | 72 (25.26)  | 4.90±1.18|         |
| T3                         | 113 (39.62) | 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           |             |          |         |
| 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 (#142) |         |
| Sex                        |                 |                 |         |
| Male                       | 174             | 91              | 83      | 0.35    |
| Female                     | 111             | 51              | 60      |         |
| Age (years)                |                 |                 |         |
| ≥70                        | 113             | 62              | 51      | 0.15    |
| <70                        | 167             | 76              | 91      |         |
| NA*                        | 5               | 4               | 1       |         |
| Depth of invasion          |                 |                 |         |
| T1                         | 13              | 8               | 5       | 0.29    |
| T2                         | 72              | 41              | 31      |         |
| T3                         | 113             | 57              | 56      |         |
| T4                         | 78              | 33              | 45      |         |
| TX                         | 9               | 3               | 6       |         |
| N classification           |                 |                 |         |
| N0                         | 94              | 51              | 43      | 0.22    |
| N1                         | 78              | 39              | 38      |         |
| N2                         | 47              | 27              | 20      |         |
| N3                         | 53              | 20              | 33      |         |
| NX                         | 13              | 5               | 8       |         |

* Not available
** Statistically significant
Table 7. Continued

| M classification | 253 | 127 | 126 | 0.04** |
|------------------|-----|-----|-----|--------|
| M0               | 18  | 5   | 13  |        |
| M1               | 14  | 10  | 4   |        |
| MX               |     |     |     |        |

| TNM stage       | 40  | 24  | 16  | 0.01** |
|-----------------|-----|-----|-----|--------|
| I                | 99  | 52  | 27  |        |
| II               | 101 | 49  | 52  |        |
| III              | 25  | 8   | 17  |        |
| IV               | 20  | 9   | 11  |        |
| NA               |     |     |     |        |

| Tumor grades    | 12  | 7   | 5   | 0.0002** |
|-----------------|-----|-----|-----|----------|
| G1-GX           | 91  | 61  | 30  |          |
| G2              | 182 | 74  | 108 |          |

| Tumor types     | 51  | 13  | 38  | 0.0002** |
|-----------------|-----|-----|-----|----------|
| Diffuse         | 95  | 58  | 37  |          |
| Intestinal      | 136 | 70  | 66  |          |
| Mixed           | 3   | 1   | 2   |          |
| NA              |     |     |     |          |

* 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* overexpression 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 cohort. 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 IncRNA 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 IncRNAs 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 IncRNAs, ecCEBPA, and UCA1 were also shown in this research. These data indicate UCA1 and ecCEBPA involvement in GC and suggest that these IncRNAs 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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