Construction of a Competitive Endogenous RNA Network in Uterine Corpus Endometrial Carcinoma

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Background:
Long non-coding RNAs (lncRNAs) affect post-transcriptional regulation by interfering with microRNAs (miRNAs), and by acting as competitive endogenous RNAs (ceRNAs). The roles and mechanisms of lncRNAs as ceRNAs in the progression and prognosis of uterine corpus endometrial carcinoma are not well understood.

Material/Methods:
We analyzed high-throughput transcriptome data downloaded from The Cancer Genome Atlas database for 548 patients with uterine corpus endometrial carcinoma, and we constructed a ceRNA network. Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes pathway analyses of differentially expressed messenger RNAs (DE-mRNAs) were performed using R software. Kaplan-Meier survival curves were generated for all RNAs in the ceRNA network.

Results:
We identified 2612 messenger RNAs (mRNAs), 1111 lncRNAs, and 187 miRNAs that were differentially expressed in uterine corpus endometrial carcinoma. We then identified mutual regulatory relationships between lncRNA-miRNA pairs and miRNA-mRNA pairs. A ceRNA regulatory network for uterine corpus endometrial carcinoma was successfully constructed, and consisted of 87 lncRNAs, 74 mRNAs, and 20 miRNAs. Nine lncRNAs, 3 miRNAs, and 22 mRNAs were associated with prognosis of uterine corpus endometrial carcinoma. We also analyzed the linear relationships between the expression of the 9 DE-lncRNAs and 22 DE-mRNAs with prognostic value.

Conclusions:
Our study showed that the lncRNAs C2orf48 and LINC00261 might be key regulators of uterine corpus endometrial carcinoma and might serve as prognostic indicators. Our study contributes to the understanding of the molecular mechanisms of uterine corpus endometrial carcinoma, and it identifies lncRNAs that might serve as prognostic markers and therapeutic targets.

MeSH Keywords: Endometrial Neoplasms • MicroRNAs • RNA, Long Noncoding

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Background

Uterine corpus cancer is the sixth most common type of cancer and the second most common gynecological malignancy in females, with an estimated 382,000 new cases and 90,000 deaths worldwide in 2018 [1]. The incidence of endometrial cancer is higher in developed countries (5.9%) than in developing countries (4.0%) [2]. Despite advances in the treatment of uterine corpus endometrial carcinoma (UCEC), including targeted therapies, the incidence of endometrial cancer is increasing. Regardless of staging or grading, cancer metastasis and recurrence are associated with poorer prognosis. The mortality rate in patients with metastasis or recurrence was significantly higher than in those without these conditions, and the overall survival time was less than 16 weeks [3]. Therefore, to improve the survival rate of patients with endometrial cancer, it is critical that novel therapeutic targets and prognostic biomarkers are identified.

The Human Genome Project confirmed that less than 2% of the human transcriptome encodes for proteins [4]. The competitive endogenous RNA (ceRNA) hypothesis posits novel and broader biological functions for the interactions between messenger RNA (mRNA) and non-coding RNA. The ceRNA hypothesis proposes a novel pattern of gene expression regulation and provides a new perspective on tumor transcriptome research. This hypothesis provides more comprehensive explanations of biological functions than other models of RNA function [5,6]. CeRNAs include long non-coding RNA (lncRNA), circulating RNA (circRNA), and pseudogenes that compete for binding to microRNA (miRNA) through miRNA response elements (MRE), resulting in mutual regulation of expression. A number of studies have shown that lncRNA-miRNA-mRNA networks play key roles in the pathogenesis and progression of breast, lung, and liver cancer, and other malignancies [7–10]. Zhou et al. demonstrated that lncRNA-RoR acts as a “sponge” for ceRNA and participates in the differentiation of endometrial cancer stem cells by interacting with miRNA-145 [11]. To date, more than 50,000 long non-coding RNA genes that were originally thought to be the “junk DNA” of the transcriptome have been identified. IncRNAs have been shown to be important markers for tumor diagnosis and prognosis. Although the study of lncRNA has progressed rapidly, the functions of most lncRNAs remain unclear.

The Cancer Genome Atlas (TCGA) is a comprehensive resource for multi-omics data including transcriptome, DNA methylation, and copy number variation for cancer genes. Advance in bioinformatics software had made it possible to construct a cancer ceRNA network using information obtained from the TCGA. Previous studies have used transcriptome data from the TCGA to construct ceRNA regulatory networks for head and neck squamous cell carcinoma [12], gastric cancer [13], papillary thyroid cancer [14], colon cancer [15], and cervical squamous cell carcinoma [16]. However, ceRNA regulatory networks for UCEC have not been constructed to date. In this study, we analyzed high-throughput transcriptome data obtained from the TCGA for 548 UCEC patients from TCGA and their corresponding clinical information.

Material and Methods

Patients and TCGA database

We downloaded level 3 RNA-seq and miRNA-seq data obtained using Illumina-HiSeq and corresponding clinical information for patients with UCEC from TCGA (https://portal.gdc.cancer.gov/). Because the data in TCGA database are open access, there was no need to obtain approval from the Ethics Committee.

Differentially expressed IncRNAs (DE-IncRNAs), miRNAs, and mRNAs analysis

Using the Ensembl database (http://www.ensembl.org), we identified miRNAs, IncRNA, miRNA in the data obtained from the TCGA. Other mRNAs and IncRNAs that could not be found using Ensembl were removed. Using the “edgeR” R software package [17], we identified differentially expressed (DE)-mRNAs, DE-IncRNA, and DE-miRNAs between UCEC and adjacent tissue, with the following statistical thresholds: |log2 fold change|≥2.0 and FDR adjusted P-value <0.01. The volcano maps and the heat maps were generated using R package “gplots”.

Construction of a ceRNA network in UCEC

To better understand the role of IncRNAs and miRNAs in patients with UCEC, we constructed a lncRNA-miRNA-mRNA interaction network. First, we predicted interactions between IncRNAs and miRNAs using the miRcode database (http://www.mircode.org/), which contains the “whole transcriptome” of GENCODE annotations, including >10,000 registered IncRNAs [18]. Next, we predicted miRNA-targeted mRNAs using the miRNA target-gene prediction project, which included miRTarbase, TargetScan, and miRDB databases. To improve the reliability of our target mRNA predictions, we used mRNAs that were present in all 3 databases to build the ceRNA network. Finally, we established matching lncRNA-miRNA and mRNA-miRNA pairs, and we constructed the competitive IncRNA-miRNA-mRNA network. The network was placed in a visual format using Cytoscape 3.5.0 software (http://www.cytoscape.org/) [19]. Finally, we analyzed the Gene Ontology (GO) enrichment of gene clusters and performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of DE-mRNAs using the “clusterprofiler” package in R [20].
We analyzed the overall survival rate associated with all RNAs in the ceRNA network using clinical data obtained from the TCGA using the "survival" R package (https://CRAN.R-project.org/, version: 3.4.3). The lncRNAs, miRNAs, and mRNAs associated with clinical prognosis were identified using univariate COX proportional hazards regression. We plotted Kaplan-Meier survival curves, and statistical significance of differences in survival was determined using the log-rank test. Values of $P<0.05$ were considered to represent statistically significant differences.

### Table 1. Clinicopathological characteristics of 548 uterine corpus endometrial carcinoma patients.

| N     | 548 |
|-------|-----|
| Age (mean SD) | 63.93 (11.14) |
| Race (%) |
| White   | 374 (68.2) |
| Black or African American | 109 (19.9) |
| Asian   | 20 (3.6) |
| Native Hawaiian or other Pacific Islander | 9 (1.6) |
| Unknown | 32 (5.7) |
| Histological type (%) |
| Endometrioid endometrial adenocarcinoma | 411 (75.0) |
| Mixed serous and endometrioid | 22 (4.0) |
| Serous endometrial adenocarcinoma | 115 (21.0) |
| Grade (%) |
| High grade | 11 (2.0) |
| G1       | 99 (18.1) |
| G2       | 122 (22.3) |
| G3       | 316 (57.7) |
| Stage (%) |
| Stage I  | 342 (62.4) |
| Stage II | 52 (9.5) |
| Stage III| 124 (22.6) |
| Stage IV | 30 (5.5) |

**Survival analysis**

We analyzed the overall survival rate associated with all RNAs in the ceRNA network using clinical data obtained from the TCGA using the "survival" R package (https://CRAN.R-project.org/, version: 3.4.3). The lncRNAs, miRNAs, and mRNAs associated with clinical prognosis were identified using univariate COX proportional hazards regression. We plotted Kaplan-Meier survival curves, and statistical significance of differences in survival was determined using the log-rank test. Values of $P<0.05$ were considered to represent statistically significant differences.

**Results**

**Patient characteristics**

RNA-sequencing data for 552 UCEC samples and 35 adjacent normal tissue samples were obtained using the TCGA. The UCEC samples were obtained from 548 patients diagnosed with UCEC using pathological analysis. The mean age of these patients was 63.9 ±11.14 years. Patients with stage I UCEC accounted for 62.4% of all patients. The pathological and clinical characteristics of the 548 patients with UCEC patients are summarized in Table 1.
Figure 2. Heat map of DE-lncRNAs in UCEC. The horizontal axis on the bottom indicates the sample name and the axis on the top indicates clustering of samples. The vertical axis on the left represents the cluster of DE-lncRNAs, and the right axis shows the names of the lncRNAs. Red indicates upregulated lncRNAs and green indicates downregulated lncRNAs. DE – differentially expressed; lncRNA – long non-coding RNA; UCEC – uterine corpus endometrial carcinoma.
Figure 3. Heat map of DE-miRNAs in UCEC. The lower horizontal axis indicates the sample name and the upper horizontal axis indicates clustering of samples. The vertical axis on the left represents the cluster of DE-miRNAs, and the right axis shows names of the miRNAs. Red indicates upregulated miRNAs and green indicates downregulated miRNAs. DE – differentially expressed; miRNA – microRNA; UCEC – uterine corpus endometrial carcinoma.
Figure 4. Heat map of DE-mRNAs in UCEC. The lower horizontal axis indicates the sample name and the upper horizontal axis indicates clustering of samples. The vertical axis on the left represents the cluster of DE-mRNAs, and the right axis shows the names of the mRNAs. Red indicates upregulated mRNAs and green indicates downregulated mRNAs. DE – differentially expressed; mRNA – messenger RNA; UCEC – uterine corpus endometrial carcinoma.
DE-mRNAs, DE-incRNAs, and DE-miRNAs

We analyzed transcriptional data from 552 tumor samples and 35 adjacent normal tissue samples and identified significant differences in the expression profiles of mRNAs, IncRNAs, and miRNAs between the UCEC and normal samples. Using thresholds of $|\log_{2}\text{FC}| \geq 2.0$ and FDR adjusted $P$-value <0.01, we determined that 2612 mRNAs, 1111 IncRNAs, and 187 miRNAs were differentially expressed. Among these, 53 miRNAs, 309 IncRNAs, and 971 mRNAs were downregulated, and 134 miRNAs, 802 IncRNAs, and 1641 mRNAs were upregulated. Volcano plots of DE-mRNAs, DE-incRNAs, and DE-miRNAs are shown in Figure 1. Heat maps of DE-mRNAs, DE-incRNAs, and DE-miRNAs obtained by cluster analysis are shown in Figures 2–4.

Construction of a ceRNA network

To further understand the functions of the DE-incRNAs, we established a regulatory ceRNA network for UCEC and generated a visual representation using Cytoscape software. We identified 477 IncRNA-miRNA regulatory pairs comprised of interactions between 27 DE-miRNAs and 89 DE-incRNAs identified using the mircode database. Following identification of IncRNA-miRNA pairs, we predicted target mRNAs based on the 27 miRNAs involved in these pairs using the mirTarbase, TargetScan, and miRDB databases. There were 962 target genes present in all 3 databases. These genes were cross-checked with the 2612 identified DE-mRNAs, resulting in selection of 74 DE-mRNAs (Figure 5). Thus, we identified 109 miRNA-mRNA regulatory pairs comprised of 20 DE-miRNAs and 74 DE-mRNAs to build the ceRNA network. The final ceRNA regulatory network for UCEC consisted of 87 DE-incRNAs, 74 DE-mRNAs, and 20 DE-miRNAs (Figure 6).

Functional prediction of DE-mRNAs

To understand the functions of DE-mRNAs, the 87 identified DE-mRNAs were subjected to GO analysis and KEGG pathway enrichment using R package “clusterProfiler”. The results showed that 7 GO enriched categories were involved in the biological processes (Figure 7). These genes were mainly enriched in cancer-related pathways such as “MicroRNAs in cancer”, “Gastric cancer”, “Prostate cancer”, “p53 signaling pathway”, “Melanoma”, “Proteoglycans in cancer”, and “Breast cancer” (Figure 8).

Survival analysis of IncRNAs, miRNAs, and mRNAs

To explore the clinical prognostic value of IncRNAs, miRNAs, and mRNAs in the ceRNA network, we investigated overall survival associated with the IncRNAs, miRNAs, and mRNAs in the ceRNA network using Kaplan-Meier curves. Nine DE-IncRNAs were significantly correlated with overall survival of patients with UCEC. Six of the DE-IncRNAs (AC110491.1, ADARB2-AS1, C2orf48, C10orf91, GLIS3-A51, and LINC00261) were inversely associated with overall survival, while 3 (AL596188.1, LINC00237, and LINCOO0261) DE-IncRNAs were directly associated with overall survival. In addition, 3 DE-miRNAs (hsa-mir-205, hsa-mir-211, and hsa-mir-425) were significantly associated with overall survival in UCEC (P<0.05) (Figure 9). Furthermore, 22 mRNAs (AKAP12, ALK, ANKRD, CCNE1, CDC25A, D, E2F1, GNAS, IGFBP5, KIF23, KPN2, MCM4, S3C1, PSAT1, RECK, RR2, SLC12A5, SOX11, TRIB3, SRTM1, RASSF2, and E2F7) were associated with overall UCEC survival. The 9 DE-IncRNAs that had clinical prognostic value interacted with 17 DE-miRNAs in the ceRNA network (Table 2). Moreover, we found that the IncRNAs C2orf48 and LINCOO261 may play an important role in the ceRNA network. The IncRNA C2orf48 may indirectly regulate 16 mRNAs with clinical prognostic value through 8 miRNAs (Table 3). In addition, the IncRNA LINCOO261 may indirectly interact with 30 mRNA-targeted miRNAs through 9 miRNAs. The R package “Hmisc” was used to analyze the linear relationships between 9 DE-IncRNAs and 22 DE-mRNAs. The results showed that the IncRNA C2orf48 positively correlated with 5 mRNAs (MCM4, CDC25A, CCNE1, KIF23, and E2F1). In contrast, the IncRNA LINCOO261 negatively correlated with 2 mRNAs (CCNE1 and ANKRD33B) (Figure 10). Our results suggest that the IncRNAs C2orf48 and LINCOO261 are key regulators of UCEC and have a prognostic role.
Figure 6. The ceRNA regulatory network for UCEC. The highlighted nodes in red indicate upregulation, and the highlighted nodes in blue indicate downregulation. LncRNAs, miRNAs and mRNAs are indicated by diamonds, square, and ellipses, respectively. ceRNA – competitive endogenous RNA; UCEC – uterine corpus endometrial carcinoma; IncRNA – long non-coding RNA, miRNA – microRNA; mRNA – messenger RNA.

Figure 7. GO biological process terms for DE-mRNAs involved in the ceRNA network. The x-axis represents the number of DE-mRNAs involved in the pathway, and the color indicates the P-value. GO – Enrichment Gene Ontology; DE – differentially expressed; mRNA – messenger RNA; ceRNA – competitive endogenous RNA.
CLINICAL RESEARCH

Ouyang D. et al.: ceRNA in UCEC
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Recent studies have shown that ceRNAs play an important role in the pathogenesis and progression of tumors characterized by abnormal transcriptome changes. We constructed a ceRNA network for UCEC based on the ceRNA hypothesis. In our ceRNA network for UCEC, 9 lncRNAs were associated with overall survival, and were considered to be biomarkers for UCEC diagnosis and prognosis. Moreover, we found that the lncRNAs C2orf48 and LINC00261 interacted to the greatest extent among lncRNAs with miRNAs in the ceRNA network. Thus, we predicted that these 2 lncRNAs may play crucial roles in the progression and prognosis of UCEC. Previous reports suggested that the lncRNA LINC00261, which may be a novel prognostic biomarker for cancer, was downregulated in various cancers such as gastric cancer [24], liver cancer [25,26], pancreatic cancer [27], non-small cell lung cancer [28], and endometrial cancer [29]. Fang et al. showed that LINC00261 can upregulate FOXO1 expression by inhibiting the miRNAs that target FOXO1, thus inhibiting proliferation, migration, and invasion of endometrial cancer cells [29]. In our study, the expression of LINC00261 increased in 552 tumor samples compared with that in 35 adjacent non-tumor samples. Interestingly, our results showed that higher expression of LINC00261 was associated with better prognosis in patients with UCEC. Li et al. constructed a ceRNA regulatory network for oral squamous cell carcinoma, and showed that 6 lncRNAs, including C2orf48, were associated with overall cancer survival [30]. Another study showed that C2orf48 could bind to RRM2 to form a chimeric transcript and high expression of RRM2-C2orf48 increased migration and invasion of nasopharyngeal carcinoma cells [31]. The role of C2orf48 in endometrial carcinoma has not been characterized. The other 7 lncRNAs identified in our study have not been previously associated with endometrial cancer; these lncRNAs may be future therapeutic targets. We also analyzed the linear relationship between the expression levels of 9 DE-lncRNAs and 22 DE-mRNAs. The results showed that C2orf48 showed a strong positive correlation with MCM4, CDC25A, CCNE1, KIF23, and E2F1 expression levels, and that LINC00261 strongly negatively correlated with CCNE1 and ANKRD33B expression levels. Based on the ceRNA regulatory network, we predicted that C2orf48 might regulate the target genes MCM4, CDC25A, CCNE1, KIF23, and E2F1 by competing with 4 key DE-miRNAs (mir-106a, mir-195, mir-216b, and mir-424). LINC00261 might regulate CCNE1 and ANKRD33B via mir-106a, mir-195, and mir-424.

Although interest in lncRNAs has increased recently, miRNAs have also attracted increased attention. Research approaches focusing on miRNA regulation are indispensable in studies of tumors. In this study, we showed that 3 miRNAs in our ceRNA network were associated with prognosis of UCEC. A previous study showed that upregulation of miR-205 was related to poor prognosis of UCEC [32]. This was consistent with

Figure 8. The KEGG pathways (P-values <0.05) of DE-mRNAs in the ceRNA regulatory network. The x-axis represents the number of DE-mRNAs involved in the pathway. The sizes of the dots indicate gene number and the color indicates the P-value. KEGG – Kyoto Encyclopedia of Genes and Genomes; DE – differentially expressed; mRNA – messenger RNA; ceRNA – competitive endogenous RNA.

## Discussion

LncRNAs are characterized by transcripts of more than 200 nucleotides in length, which regulate DNA methylation, histone modification, and chromatin remodeling, and are precursors to miRNA [21]. Recent reports suggest that lncRNAs are involved in the pathogenesis of different types of cancer, including endometrial cancer. Sun et al. identified 5 lncRNAs in UCEC (CTB-S122.1, FLJ27354, VIM-AS1, RP11-27514.4, and RP11-229P13.20) that were significantly related to tumor progression and patient outcomes [22]. These lncRNAs provided a signature that was an independent predictor of prognosis, as determined using multivariate Cox regression analysis. Another study showed that the lncRNA HOTAIR regulated estrogen-induced endometrial cancer metastasis through the miR-646/NPM1 axis [23].

Recent studies have shown that ceRNAs play an important role in the pathogenesis and progression of tumors characterized by abnormal transcriptome changes. We constructed a ceRNA network for UCEC based on the ceRNA hypothesis. In our ceRNA network for UCEC, 9 lncRNAs were associated with overall survival, and were considered to be biomarkers for UCEC diagnosis and prognosis. Moreover, we found that the lncRNAs C2orf48 and LINC00261 interacted to the greatest extent among lncRNAs with miRNAs in the ceRNA network. Thus, we predicted that these 2 lncRNAs may play crucial roles in the progression and prognosis of UCEC. Previous reports suggested that the lncRNA LINC00261, which may be a novel prognostic biomarker for cancer, was downregulated in various cancers such as gastric cancer [24], liver cancer [25,26], pancreatic cancer [27], non-small cell lung cancer [28], and endometrial cancer [29]. Fang et al. showed that LINC00261 can upregulate FOXO1 expression by inhibiting the miRNAs that target FOXO1, thus inhibiting proliferation, migration, and invasion of endometrial cancer cells [29]. In our study, the expression of LINC00261 increased in 552 tumor samples compared with that in 35 adjacent non-tumor samples. Interestingly, our results showed that higher expression of LINC00261 was associated with better prognosis in patients with UCEC. Li et al. constructed a ceRNA regulatory network for oral squamous cell carcinoma, and showed that 6 lncRNAs, including C2orf48, were associated with overall cancer survival [30]. Another study showed that C2orf48 could bind to RRM2 to form a chimeric transcript and high expression of RRM2-C2orf48 increased migration and invasion of nasopharyngeal carcinoma cells [31]. The role of C2orf48 in endometrial carcinoma has not been characterized. The other 7 lncRNAs identified in our study have not been previously associated with endometrial cancer; these lncRNAs may be future therapeutic targets. We also analyzed the linear relationship between the expression levels of 9 DE-lncRNAs and 22 DE-mRNAs. The results showed that C2orf48 showed a strong positive correlation with MCM4, CDC25A, CCNE1, KIF23, and E2F1 expression levels, and that LINC00261 strongly negatively correlated with CCNE1 and ANKRD33B expression levels. Based on the ceRNA regulatory network, we predicted that C2orf48 might regulate the target genes MCM4, CDC25A, CCNE1, KIF23, and E2F1 by competing with 4 key DE-miRNAs (mir-106a, mir-195, mir-216b, and mir-424). LINC00261 might regulate CCNE1 and ANKRD33B via mir-106a, mir-195, and mir-424.

Although interest in lncRNAs has increased recently, miRNAs have also attracted increased attention. Research approaches focusing on miRNA regulation are indispensable in studies of tumors. In this study, we showed that 3 miRNAs in our ceRNA network were associated with prognosis of UCEC. A previous study showed that upregulation of miR-205 was related to poor prognosis of UCEC [32]. This was consistent with
Figure 9. Kaplan-Meier curve analyses of DE-lncRNAs, DE-miRNAs, and overall survival rate in patients with UCEC. DE – differentially expressed; lncRNA – long non-coding RNA, UCEC – uterine corpus endometrial carcinoma.
Table 2. The 9 DE-lncRNAs interact with the 17 DE-miRNAs.

| lncRNA       | miRNA                                                                 |
|--------------|------------------------------------------------------------------------|
| AC110491.1   | mir-140, mir-141, mir-200a, mir-143, mir-182, mir-429, mir-204, mir-211, mir-205, mir-216b, mir-425 |
| ADARB2-AS1   | mir-195, mir-424, mir-205, mir-216b                                       |
| ALS61881.1   | mir-205                                                                |
| C10orf91     | mir-106a, mir-140, mir-143, mir-429, mir-204, mir-211, mir-122          |
| GLI53-AS1    | mir-140                                                                |
| LINC00237    | mir-143, mir-205                                                        |
| LINC00261    | mir-140, mir-143, mir-145, mir-182, mir-183, mir-429, mir-204, mir-211, mir-216b |
| LINC00491    | mir-145, mir-429, mir-204, mir-211, hsa-mir-216b, mir-122               |

DE – differentially expressed; lncRNA – long non-coding RNA, miRNA – microRNA.

Table 3. The 8 DE-miRNAs Interact with the 16 DE-mRNAs.

| miRNA       | mRNA                                                                      |
|-------------|---------------------------------------------------------------------------|
| mir-106a    | ANKR3D33B, E2F1, KIF23, KPN2A, RRM2                                        |
| mir-143     | IGFBP5                                                                    |
| mir-145     | DDC                                                                       |
| mir-182     | NR3C1, RECK                                                               |
| mir-183     | AKAP12, NR3C1                                                             |
| mir-195     | CCNE1, CDC25A, KIF23, PSAT1, RECK, RASSF2, E2F7                           |
| mir-216b    | MCM4                                                                      |
| mir-424     | CCNE1, CDC25A, GNAL, KIF23, PSAT1, RECK, RASSF2, E2F7                     |

DE – differentially expressed; miRNA – microRNA; mRNA – messenger RNA.

Figure 10. Linear regression of C2orf48 versus MCM4, CDC25A, KIF23, E2F1, and LINC00261 versus expression level of CCNE1 and ANKR3D33B. Dashed lines represent 95% confidence intervals.
our results. Xu et al. showed that miR-211 suppressed cervical cancer cell invasion and epithelial-to-mesenchymal transition (EMT) by targeting MUC4 [33]. Our results showed that the prognosis for patients with UCEC with high expression of miR-211 and miR-425 was poor. However, further experiments are needed to confirm the prognostic value of these miRNAs in endometrial cancer. Kong et al. reported that the IncRNA PVT1 promoted proliferation, migration, and invasion of endometrial carcinoma cells via miR-195-5p [34]. Another study demonstrated that knockdown of CCAT2 suppressed endometrial cancer cell growth and metastasis by targeting miR-216b [35]. In addition, the miRNA-424/E2F7 axis might inhibit the malignancy of endometrial cancer [36].

KEGG pathway enrichment analysis showed that mRNAs involved in the ceRNA network were mainly enriched in “microRNAs in cancer”, “Melanoma”, “Gastric cancer”, “Prostate cancer”, “p53 signaling pathway”, and “Proteoglycans in cancer” pathways, which are related to cancer. Among these miRNA target-genes, genes related to LINCO0261, C2orf48, and prognosis of endometrial cancer included MCM4, CDC25A, CCNE1, KIF23, E2F1, and ANKR3D3B. We found that these genes played a key role in the development and progression of cancer. In endometrial cancer cell lines, CYP1B1 knockdown resulted in the upregulation of MCM4 [37]. In addition, miR-449a might suppress tumor development by targeting CDC25A in endometrial cancer [38]. In a pan-cancer study, increased expression of CCNE1 was observed in African Americans with cancer types with higher chromosomal instability [39]. In addition, Mints et al. showed that high expression of free E2F1 might be a good prognostic marker for endometrial cancer [40]. Several studies have shown that KIF23 has good prognostic value for various cancers, including non-small cell lung cancer [41], pancreatic carcinoma [42], kidney renal clear cell carcinoma [43], and breast cancer [44].

The limitations of our study were as follows. First, the ceRNA hypothesis has been studied extensively, but remains controversial. Several studies have shown that expression profile changes in most individual transcriptomes are not be affected by miRNA activity [45]. Our ceRNA regulatory network for UCEC was constructed based on this hypothesis using a bioinformatics approach. Further experiments should focus on verification of these key interactions, particularly the IncRNAs identified in the present study. Second, the 9 IncRNAs screened from TCGA, a database that includes mostly Caucasian individuals, require further evaluation to understand whether the pathogenic mechanisms in Asian or other races are the same.

Conclusions
We identified DE-IncRNA, DE-miRNA, and DE-mRNA in tumor and adjacent normal tissue samples in a large cohort of patients with UCEC using data obtained from the TCGA. A regulatory ceRNA network was constructed, and several IncRNAs with potential clinical prognostic value were identified. Our study advances the understanding of the molecular mechanisms of UCEC and promotes the discovery of novel ceRNAs that can serve as prognostic markers and therapeutic targets.

Conflicts of interests
None.

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