Identification of LINC01503 as Biomarker Regulated by CTBP1 with Prognostic and Diagnostic Role in Epithelial Ovarian Cancer

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Abstract

Background: Epithelial ovarian cancer (EOC) is a disease with high morbidity and mortality worldwide, which is seriously harmful to female health. LncRNA has an important relationship with the occurrence and development of tumors. Hence, the investigation of the underlying mechanism between LncRNA and EOC is of great importance.

Results: In this study, we found that LINC01503 was highly expressed in EOC with a poor prognosis based on microarray datasets GSE119056 and GSE135886 obtained from Gene Expression Omnibus (GEO) database, and this result was verified by RT-qPCR. The database lncBase Predicted v.2 and starBase v2.0 were used to predict the targeted relationship of lncRNA-miRNA-mRNA, then the ceRNA network was established by Cytoscape software. Following, the expression and overall survival (OS) analysis of key lncRNAs were analyzed by GEPIA and Kaplan-Meier plotter database. Gene Ontology (GO) functional enrichment analysis was performed by DAVID database and enriched two cancer related biological processes (BP) that response to endoplasmic reticulum stress and IRE1-mediated unfolded protein. Moreover, we verified that LINC01503 was an oncogene regulated by C-terminal binding protein 1 (CTBP1) to promote cell proliferation, migration and inhibited cell apoptosis in ovarian cancer.

Conclusion: In conclusion, these results identified LINC01503 as a potential gene for EOC diagnosis and prognosis.

1. Introduction

Epithelial ovarian cancer (EOC) was generally found at an advanced stage, and because of its high morbidity and high mortality, it was a threat to the health of women [1]. EOC accounts for more than 90% of ovarian cancer, in addition, high-grade serous ovarian cancer was the most common histologic subtype of EOC and account for over 70% of EOC [2–4]. Although advances in science have improved the survival rate of many cancers, the survival rate of EOC has not improved significantly[5]. EOC was a heterogeneous disease, including tumors of different types of tissue, grade and microenvironmental characteristics, all of which contribute to the response and outcome of treatment[1, 2, 6]. Improving the survival of patients with ovarian cancer also relies on diagnosis and prognosis.

The endoplasmic reticulum (ER) is the site of synthesis and folding of secretory and membrane bound proteins. ER regulates protein synthesis, protein folding and trafficking, cellular responses to stress and intracellular calcium ($Ca^{(2+)}$) levels.[7]. ER stress response could be important for the growth and development of tumors under stressful growth conditions such as hypoxia or glucose deprivation, which are commonly encountered by most solid tumors[8]. ER stress response is also activated in hypoxic or nutrient deprived tumors [9]. Therefore, ER stress response mechanisms could be targeted to overcome chemoresistance in EOC[10].

In recent years, non-coding RNA has played a vital role in cancer and has been confirmed by many studies. In molecular biology, ceRNA effect other RNA transcripts by competing for shared microRNAs (miRNAs) [11]. CeRNA can be regarded as a balance, it will lead to the disturbance of life activities and cause the occurrence of diseases when the balance is broken [12–14]. Although lncRNA had an important influence on the occurrence and development of different cancers, the scientific research on the biomolecular mechanism of lncRNA in tumors was still unclear[15]. Just as knockdown of lncRNA Pvt1 reduced the ability of G-MDSCs to delay tumor progression in tumor-bearing mice in vivo [16]. HULLK was a novel lncRNA located in the LCK gene and was a significant positive correlation between HULLK high expression and prostate cancer (PCa), the shRNA targeting HULLK significantly reduces the growth of PCa cells [17]. Moreover, previous studies have shown that lncRNA MALAT1 inhibited tumor metastasis in breast cancer, and the expression level of MALAT1 was inversely proportional to the progression and metastatic ability of breast cancer [18]. In addition, lncRNA has also been studied in ovarian cancer, and revealed the important role of lncRNA in ovarian cancer [14, 19].

In this study, we acquired the genes expression profile of EOC through Gene Expression Omnibus (GEO) database, which includes mRNAs, miRNAs and lncRNAs. The ceRNA network of lncRNA-miRNA-mRNA was constructed by bioinformatics analysis to search key lncRNAs related to EOC. Prognostic analysis of these key lncRNAs were performed based on the clinical

data of Cancer Genome Atlas (TCGA), and found that LINC01503 can be used as an indicator of the prognosis of EOC. Finally, biological processes ER stress related to the occurrence of cancer were enriched by GO. This study provides a mechanism of EOC development and new insights for diagnosis and prognosis.

2. Results

2.1 Screening the DEGs in EOC

In this study, we identified differentially expressed lncRNAs, mRNAs and miRNAs between in ovarian tumors and adjacent normal tissues. Firstly, we analyzed the differentially expressed lncRNA of the normal and cancer samples in the datasets GSE119054 and GSE13588. Volcano plot analysis showed that 759 and 605 DE-lncRNAs were separately identified in the datasets as shown in Fig. 1A and 1B. The Venn diagram showed that GSE119054 and GSE13588 have a total of 59 common DE-lncRNAs (Fig. 1C). We performed cluster analysis on these 59 DE-lncRNAs and found that there were 51 lncRNAs with the same trend in the two datasets, of which 6 up-regulated lncRNAs and 45 down-regulated lncRNAs (Fig. 1D).

Then, we conducted differential expression analysis of mRNAs (Fig. 2A and 2B), the volcano plot confirmed 1749 and 4945 differentially expressed mRNAs of the datasets GSE119054 and GSE13588, respectively. The Venn diagram demonstrated that there were 615 differentially expressed mRNAs in common between GSE119054 and GSE13588, of which 503 differential mRNAs have the same trend (Fig. 2D, 2E). In addition, GSE119055 was used to analyze the expression of differentially expressed miRNAs, and 54 differentially expressed miRNAs were identified as shown in Fig. 2C.

2.2 Construction of ceRNA networks

In order to better understand the biological role and impact of lncRNAs in EOC, we constructed a ceRNA analysis to search for key lncRNAs which affect the occurrence and development of EOC. The target relationship was predicted by searching the databases lncBase Predicted v.2 and starBase v2.0, and the ceRNA network was constructed through Cytoscape. According to the DE-lncRNAs, mRNAs and miRNAs, the final determined lncRNA-miRNA-mRNA network as shown in Fig. 3. The constructed network of ceRNA contains an up-regulation network of lncRNAs (Fig. 3A) and a down-regulation network of lncRNAs (Fig. 3B), which includes 20 lncRNAs, 15 miRNAs and 77 mRNAs. The lncRNA up-regulation network contains 3 up-regulated lncRNAs, 60 up-regulated mRNAs and 12 down-regulated miRNAs. The down-regulation network of lncRNA contains 17 down-regulated lncRNAs, 17 down-regulated mRNAs and 3 up-regulated miRNAs.

2.3 The prognosis of LINC01503 and RT-qPCR verification

Then, we analyzed all the lncRNAs expreession of ceRNA network used the GEPIA database, and found that the LINC01503 was significantly high expression in EOC as shown in the Fig. 4A. To evaluate whether the expression of LINC01503 has an effect on the OS of EOC patients, the Kaplan-Meier plotter database was used to analyze the data of EOC. The result showed high expression LINC01503 group had worse OS (HR = 1.48, p-value = 6.2e-05) (Fig. 4B).

Next, a total of 25 EOC samples and adjacent cancer samples were enrolled as a validation cohort. RT-qPCR technology was used to confirm the differential expression levels from participant's tissues. Consistent with the microarray data, LINC01503 was significantly upregulated (Fig. 4C) between controls and EOC. To assess the potential value of confirmed LINC01503 for EOC diagnosis, we further performed ROC curve analysis. We found that ROC curve of LINC01503 showed a distinguishing efficiency with an AUC value of 0.828 (95% CI: 0.717–0.93, **p < 0.01) (Fig. 4D), with the best cut-off value of 8.5, the sensitivity was 56% and specificity92%. which indicated that LINC01503 could be a potential biomarker for EOC diagnosis.

2.4 Enrichment analysis of DE- mRNAs related to LINC01503

In order to understand the molecular mechanism of LINC01503 in EOC. We used the Cytoscape to construct a ceRNA subnetwork about LINC01503, and LINC01503 could competitively adsorbed has-miR-130a-3p. There are 25 mRNAs targeted by has-miR-130a-3p as shown in Fig. 5A. Furthermore, GO enrichment analysis of the 25 mRNAs was performed to explore the potential biological processes of LINC01503 in EOC. Enrichment analysis enriched 10 biological processes that were cellular

response to external stimulus, endoplasmic reticulum stress, neuron death, neuron apoptotic process and extracellular stimulus (Fig. 5B, Table 2). Additionally, we found that response to endoplasmic reticulum stress and IRE1-mediated unfolded protein response were related to the occurrence and development of cancer.

Table 2
The top 10 enriched GO-BP terms

ID	Description	p-value	geneID
GO:0036498	IRE1-mediated unfolded protein response	4.12E-06	BAK1/DNAJB11/HSPA5/SRPRB/TPP1
GO:0071496	cellular response to external stimulus	4.74E-06	AIFM1/BAK1/DSC2/GABARAPL1/GCLC/HSPA5/ITGA4/RALB/SLC2A1
GO:0034976	response to endoplasmic reticulum stress	1.11E-05	AIFM1/BAK1/DNAJB11/HSPA5/ITPR1/SRPRB/TMX1/TPP1
GO:0051402	neuron apoptotic process	3.12E-05	AIFM1/BTG2/GCLC/HSPA5/MECP2/RB1/TNFRSF21
GO:0070997	neuron death	4.64E-05	AIFM1/BTG2/DHCR24/GCLC/HSPA5/MECP2/RB1/TNFRSF21
GO:0031668	cellular response to extracellular stimulus	6.44E-05	AIFM1/DSC2/GABARAPL1/HSPA5/ITGA4/RALB/SLC2A1
GO:0043496	regulation of protein homodimerization activity	7.39E-05	BAK1/HSPA5/ITGA4
GO:0090074	negative regulation of protein homodimerization activity	8.27E-05	HSPA5/ITGA4
GO:0030968	endoplasmic reticulum unfolded protein response	9.10E-05	BAK1/DNAJB11/HSPA5/SRPRB/TPP1
GO:0034620	cellular response to unfolded protein	0.0001806	BAK1/DNAJB11/HSPA5/SRPRB/TPP1

2.5 LINCRNA01503 is regulated by CTBP1 in in ovarian cancer.

The results above indicated that LINC01503 is oncogene with prognosis value. In the next step, we carried out the cellular experiment for investigation of the underlying mechanism. Three shRNAs were designed to intervene the expression of LINCRNA01503 in OVCAR-3 or SK-OV-3 cell lines. As shown in Fig. 6A, the LINCRNA01503 shRNAs significantly decreased the expression of LINCRNA01503 in both OVCAR-3 or SK-OV-3 cell lines compared to control group, especially LINCRNA01503 shRNA-1. Hence, the LINCRNA01503 shRNA-1 was selected for the further experiments. The cell numbers in the group treated with LINCRNA01503 shRNA-1 were significantly decreased (Fig. 6B). The cell apoptosis was significantly enhanced after treating with LINCRNA01503 shRNA-1, suggesting that LINCRNA01503 inhibited cell death (Fig. 6C). Wound scratch assay results indicated that LINCRNA01503 promoted cell migration (Figs. 6D). Furthermore, we conducted ChIP sequencing in SK-OV-3 cell lines to further investigate the underlying mechanism of LINCRNA01503. The C-terminal binding protein 1 (CTBP-1), a transcriptional corepressor of oncogenic processes s [20], showed significantly different peaks between the two groups (Fig. 6E). Luciferase reporter gene experiment verified the tight binding of LINCRNA01503 and CTBP-1 (Fig. 6F). Also, the

relative expression of LINCRNA01503 and CTBP-1 in the SK-OV-3 cell lines treated with CTBP-1 shRNA were significantly decreased compare to control group. These results revealed that LINCRNA01503 is regulated by CTBP1 in in ovarian cancer.

3. Discussion

The most common type of ovarian cancer was epithelial ovarian cancer (EOC). The cause of ovarian cancer was very complicated, which may be caused by factors such as family inheritance, obesity, low immunity, environmental pollution, unreasonable diet, chronic inflammatory stimulation, and benign ovarian cancer [21–27]. Nowadays, with the development of medical treatment, there were many treatments of ovarian cancer [28, 29]. For example, neoadjuvant chemotherapy (NACT) has significantly reduced mortality in the treatment of advanced EOC after diagnosis [30]. In addition, the rise of targeted therapy [31] and immunotherapy also brings more hope to EOC [32]. However, EOC is a highly fatal malignant tumor and is usually diagnosed only at an advanced stage, which will lose the best treatment time [33]. Early diagnosis of EOC has always been a major challenge. Therefore, it is a great significance that searching for specific targets and biomarkers for diagnosis and prognosis of EOC.

LncRNA is considered as the main component of the ceRNA networks, because it regulates the expression of mRNA by absorbing miRNA as a sponge [34, 35]. Therefore, lncRNA obtained increased attention in human cancers as its multifarious function [36–38]. Previous researches showed that lncRNAs played a critical role in the progression of many cancers. For instance, lncRNAs have been studied in lung cancer [39], liver cancer [40], bladder cancer [41], prostate cancer [42] and breast cancer [43]. LncRNAs have also been studied in ovarian cancer, but it is little known about the crosstalk between mRNAs, miRNAs, and lncRNAs, looking for key lncRNAs and exploring the molecular mechanisms related to EOC are an urgent work. In our study, the expression levels and prognosis of lncRNAs in ceRNA network were analyzed through the database GEPIA and Kaplan-Meier plotter, and it was confirmed that LINC01503 was differentially expressed in normal tissues and EOC, and high expression of LINC01503 had an adverse effect on prognosis. In addition, the RT-qPCR experiment further verified our analysis results.

CeRNA network plays an important role to discover biomarkers for clinical prognosis and diagnosis in cancer [44, 45]. Studies have revealed that the activation of the STARD13-correlated ceRNA network is negatively correlated with breast cancer YAP/TAZ activity [46]. In our research, we obtained mRNAs, lncRNAs and miRNAs expression profiles from the GEO database. Next, we constructed ceRNA network of differentially expressed lncRNA-miRNA-mRNA to explore key lncRNAs associated with EOC diagnosis and prognosis. Then it was found that LINC01503 affected the OS and prognosis of EOC patients. Subsequently, we explore the molecular mechanism of LINC01503 through the GO enrichment analyzed the competitive mRNAs of LINC01503. In the 10 biological processes enriched, which response to endoplasmic reticulum stress and IRE1-mediated unfolded protein response have been reported to be related to the occurrence of cancer. Interaction between ER stress contributes to the occurrence and development of various types of cancer[47] Zhang et. al indicated that Angiotensin II promotes ovarian cancer spheroid formation and metastasis by upregulation of lipid desaturation and suppression of ER stress[48]. As the tumor microenvironment is affected by oxidative stress, when the balance between endoplasmic reticulum folding and the degradation of transfer proteins and misfolded proteins is broken, the endoplasmic reticulum (ER) stress response occurs [49]. PERK attenuates IRE1 via RPAP2 to abort failed ER-stress adaptation and trigger apoptosis [50].

The LINC01503 have been reported as an oncogene in non-small cell lung cancer[51], Gastric Cardia Adenocarcinoma[52], cervical cancer[53], etc. We verified that the LINC01503 promote r cell proliferation, migration and inhibited cell apoptosis in ovarian cancer. In the next step, we verified its underlying mechanism. The CTBP-1 is well-known transcriptional corepressors of oncogenic processes [20]. There are some researches revealed the important role of CTBP-1 in EOC before. For example, Ding et al reported that CTBP determines ovarian cancer cell fate through repression of death receptors[54]. He et al. indicated that CtBP-1 differentially regulate genomic stability and DNA repair pathway in high-grade serous ovarian cancer cell[55]. It was the first time to report the regulation role of CTBP1 on LINC01503 in EOC, which was provided the molecular mechanisms for further investigation.

4. Conclusion

In the current study, we constructed the ceRNA network based on the data in the GEO database, and found a highly expressed LINC01503 with a poor prognosis was related with endoplasmic reticulum stress in EOC, which verified by RT-qPCR. Moreover, we verified that LINC01503 was an oncogene regulated by CTBP1 to promote cell proliferation, migration and inhibited cell apoptosis in ovarian cancer. This study provides reference value for diagnosis and prognosis of LINC01503 in clinical. However, the molecular mechanisms of LINC01503 in EOC in vivo is needed further study.

5. Materials And Methods

5.1 Patients

A total of 25 pairs of Epithelial ovarian cancer and corresponding adjacent non-tumor specimens were collected from Henan Provincial People's Hospital (Zhengzhou, China). The research protocol for this research was approved by the Ethics Committee of Henan Provincial People's Hospital. Informed consent was obtained from all participants. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards

5.2 Data acquisition

This study integrated analysis two datasets GSE119056 [56] and GSE135886 [57], which were from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). GSE119056 contains 12 cases of ovarian malignant tumor tissues and 6 cases of ovarian normal tissues, and GSE119056 was divided into two subseries GSE119054 and GSE119055. GSE119054 was the dataset of mRNA and long noncoding RNA expression profile of EOC and normal samples, and GSE119055 was the dataset of microRNA expression profiling of EOC tissues and normal ovaries. GSE135886 contains 6 normal ovarian samples, 6 high-grade serous ovarian carcinoma samples and 6 low-grade serous ovarian carcinoma samples. 6 cases of high-grade serous EOC and 6 cases normal EOC were selected as the research object by our analysis.

5.3 Data processing

The names and annotation information of mRNAs, lncRNAs and miRNAs of EOC in GSE119056 and GSE135886 were re-annotated by the latest transcript sequence of Ensembl database (http://asia.ensembl.org/index.html) [58]. The R package preprocessCore was used to perform quantile normalization on the data, log2 transformation was performed on the gene expression data. The average RNA expression was used when duplicate data was found, and low-abundance microarray data were removed.

5.4 Identification of differentially expressed genes (DEGs)

We identified the differentially expressed lncRNAs and mRNAs in the datasets GSE119054 and GSE135886. Similarly, we identified the differentially expressed miRNAs in GSE119055. All of the DEGs were performed by limma package (Version 3.38.3; http://bioconductor.org/packages/3.8/bioc/html/limma.html). Genes with an p-value < 0.05 and |log2FC|>=1 were assigned as differentially expressed. The p-value adjusted to false discovery rate (FDR) by multitest package (Version 2.44.0; http://bioconductor.org/packages/release/bioc/html/multtest.html). The pheatmap R package (Version 1.0.12; https://cran.r-project.org/web/packages/pheatmap/) was used to perform hierarchical cluster analysis on EOC and normal samples. In addition, all of the DEGs were analyzed in the various datasets by Venn analysis to detect the intersection genes between the normal and ovarian malignant tumor samples.

5.5 Construction of the ceRNA network

The lncBase Predicted v.2 database (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=lncbasev2/index-predicted) was used to predict the interaction relationship between differential expression lncRNA and miRNA [59]. The interaction relationship between differential expression miRNA and mRNA were predicted by starBase v2.0 database (http://starbase.sysu.edu.cn/) [60]. The starBase v2.0 through the five software: targetScanSites, picTarSites, RNA22Sites,

PITASites, miRandaSites to predict miRNA target genes. Next, the lncRNA-miRNA-mRNA interaction network was constructed using the Cytoscape software (https://cytoscape.org/).

5.6 Function enrichment analysis of differential genes in ceRNA network

GO enrichment analysis of the differential genes in the ceRNA network was analyzed by the online software DAVID (https://david.ncifcrf.gov/) [61]. Defined statistical significance with FDR < 0.05.

5.7 Cell line

Human ovarian cancer cell lines OVCAR3 and SKOV3 (ATCC, USA) were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/L streptomycin. The cells were placed in the incubator at 37°C and 5% CO_2 for static culture.

5.8 Cell transfection LINC01503

Ovarian cancer cells (SKOV3 and OVCAR3) in the logarithmic growth phase were inoculated into 6-well cell culture plates. When the confluence of cells reached 30–40%, the transfection was performed according to the instructions of the Lipofectamine 2000 kit (Invitrogen; Thermo Fisher Scientific, Inc.), and short hairpin (sh)RNAs targeting LINC01503 and their corresponding controls were transfected separately. After 24 h of transfection, the medium was replaced with fresh medium. The transfected shRNAs were synthesized by Sangong Co. Ltd (Shanghai). The sequencings were shown in Table 1.

Table 1
Specific RNAs primers for quantitative qRT-PCR analysis

Gene name	Sequence
GAPDH	F: GCCAAGGCTGTGGGCAAGGT
	R: TCTCCAGGCGGCACGTCAGA
LINC01503	F: CTTTCCCTGAGGACCATCTG
	R: CAAAATCCGGTCTTTCTGGA
CTBP-1	F: TACCATGGGGAGATCTGGCA
	R: AGAGGCTTGAGAGTGCACAC
LINC01503-shRNA1	GCTCGGAATACCCACCTTTCT
LINC01503-shRNA2	GCCTCTGACAAGTGTGTACCT
LINC01503-shRNA3	GGAATACCCACCTTTCTGGTA
CTBP-1-shRNA	GCATGTGCTCGCTGAACAAAC

5.9 Cell-Counting-Kit-8 (CCK-8)

Cell proliferation was assessed by Cell Counting Kit-8 assay (Sangon, Shanghai). Cells (1×10^3) were seeded into 96-well plates and incubated at 37°C for 24 h before transfection. CCK-8 solution (10 μl) was added to each well 48 h after transfection. After 2 h of incubation at 37°C, the absorbance at 450 nM was measured using Spectra Max 250 spectrophotometer (Molecular Devices, USA). Triplicate independent experiments were performed.

5.10 Apoptosis assay

For apoptosis assay, cells were stained by propidium iodine/Annexin V-FITC staining (BD Biosciences) then analyzed by flow cytometry FACS Calibur instrument (BD Biosciences) according to the manufacturer's instructions.

5.11 Wound healing assay

The OVCAR3 and SKOV3 Cells (2×10^5/well were plated into 12-well plates until the cells reached 90% confluency. The fused monolayer cells were then scratched with a pipette tip (100 μl), and the exfoliated cells were washed gently with PBS. Subsequently, the cells were cultured in a serum-free medium for 48 h. Using an optic microscope (Leica), the images at 0 and 48 h were captured with ×100 magnification to evaluate cell migration.

5.12 Chromatin immunoprecipitation (ChIP) analysis

The SK-OV-3 cells were treated with 1% formaldehyde and then quenched with glycine for 5 min at room temperature. ChIP assays were performed using a chromatin IP kit (Cell Signaling Technology, Danvers, MA, United States) according to the manufacturer's instructions. The analysis was conducted with peak caller MACS2[62].

5.13 Luciferase reporter assay

Luciferase reporter vector with the full length of the 3'- UTR of LINC01503 (LINC01503 pro WT: CCCCCTGAAGGCTCTGCCTGGAAGGAGCGAAGGGGTTAAGTGTTTCTGGC) and the mutant version (LINC01503 pro MUT CCCCCTGAATTACGGCAACCTTTCCTCGATTCCAACCTTCGCAAACTGGC) were constructed. Luciferase reporter vector with CTBP-1 shRNA was transfected into SK-OV-3cells. After 48 h of incubation, the firefly and Renilla luciferase activities were quantified with a dual-luciferase reporter assay (Promega, USA).

5.14 Real-time quantitative reverse transcription PCR (RT-qPCR)

Total RNA was extracted using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. RT-qPCR was performed using the SYBR Green qPCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. GAPDH was used as the internal control. The sequences of specific primers used in this study are listed in Table 1.

5.15 Survival analysis

In order to evaluate the prognosis of differential expression lncRNAs combining the clinical data of EOC patients in Kaplan-Meier plotter. We used GEPIA 2 (http://gepia2.cancer-pku.cn/#index) to analyze differentially expressed lncRNA in EOC tumor tissues and adjacent tissues, and obtained the prognostic survival curves (p-value < 0.05 and $|\log_2 FC| \geq 1$ as the cut-off criterion).

6. Abbreviations

Abbreviations	Description
EOC	Epithelial ovarian cancer
GEO	Gene Expression Omnibus
OS	Overall survival
GO	Gene Ontology
CTBP-1	C-terminal binding protein 1
BP	Biological processes
ER	The endoplasmic reticulum
miRNAs	microRNAs
TCGA	clinical data of Cancer Genome Atlas
DEGs	differentially expressed genes
FDR	false discovery rate ()
shRNAs	short hairpin (sh) RNAs

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yanchun Wang and Xiaohua Li conceived and designed the experiments. Zheng Wei drafted the manuscript. Junping Zhang and Xuemei Wang analyzed the data. Yanchun Wang prepared figures and/or tables. Xiaohua Li approved the final draft. All authors read and approved the final manuscript.

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Figures

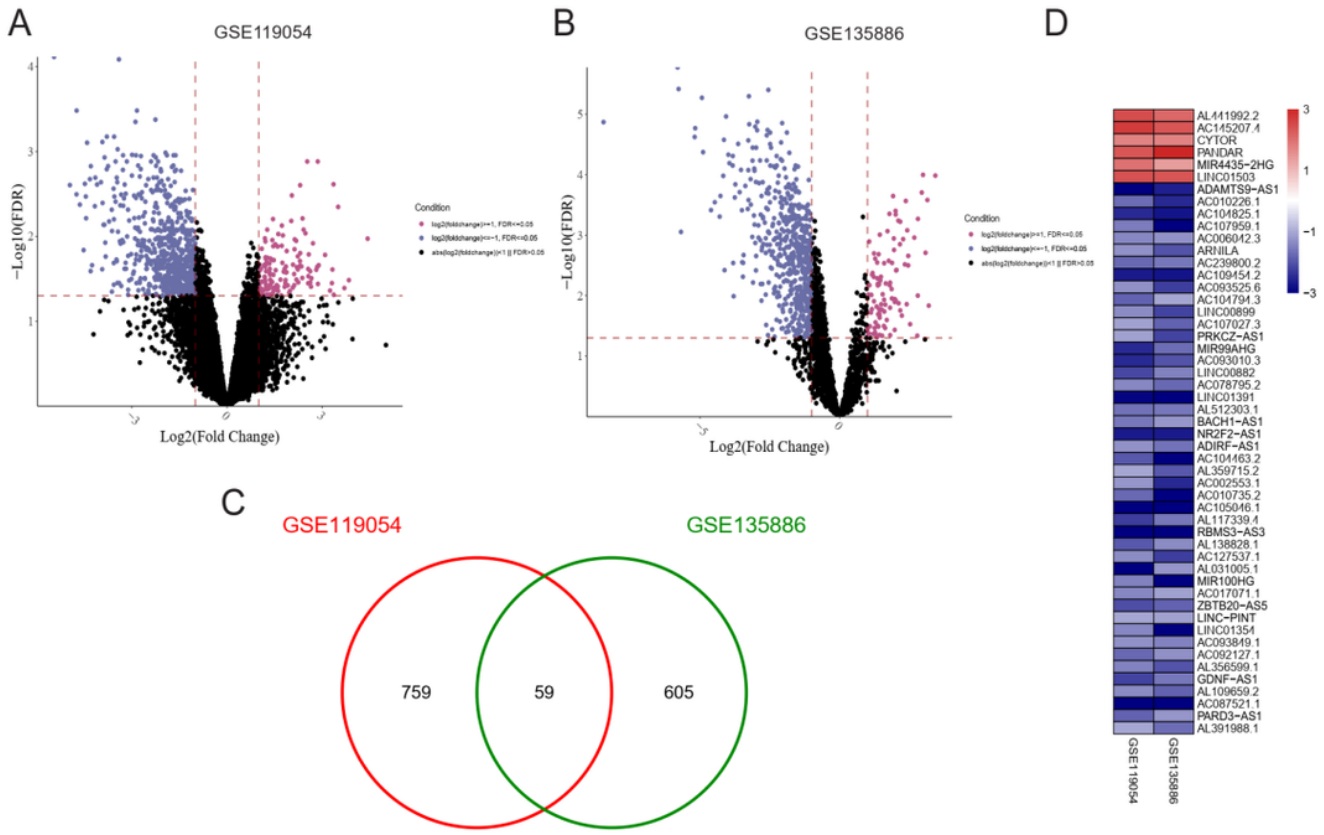

Figure 1

Differential lncRNAs expression in EOC. (A) Volcano plot of differentially expressed lncRNAs in the dataset GSE119054. (B) Volcano plot of differentially expressed lncRNAs in the dataset GSE135886. (C) Venn analyses of differential expressed lncRNAs. (D) Heatmap cluster analysis of differential lncRNAs, red represents up-regulated genes and blue represents up-regulated genes.

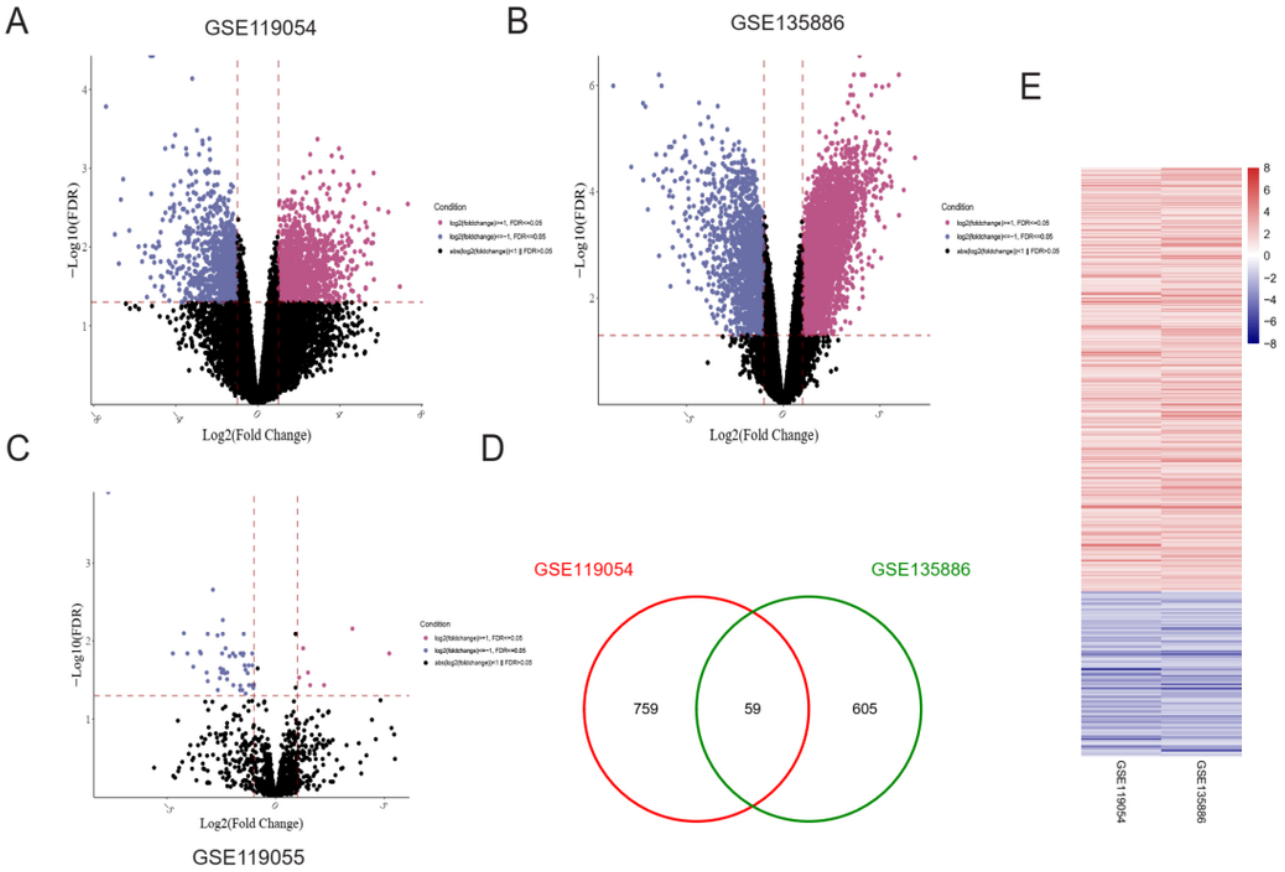

Figure 2

Differential mRNAs and miRNAs expression in EOC. (A) Volcano plot of differentially expressed mRNAs in the dataset GSE119054. (B) Volcano plot of differentially expressed mRNAs in the dataset GSE135886. (C) Volcano plot of differentially expressed miRNAs in the dataset GSE119055. (D) Venn analysis of differential expressed mRNAs. (E) Heatmap cluster analysis of differential mRNAs, red represents up-regulated genes and blue represents up-regulated genes.

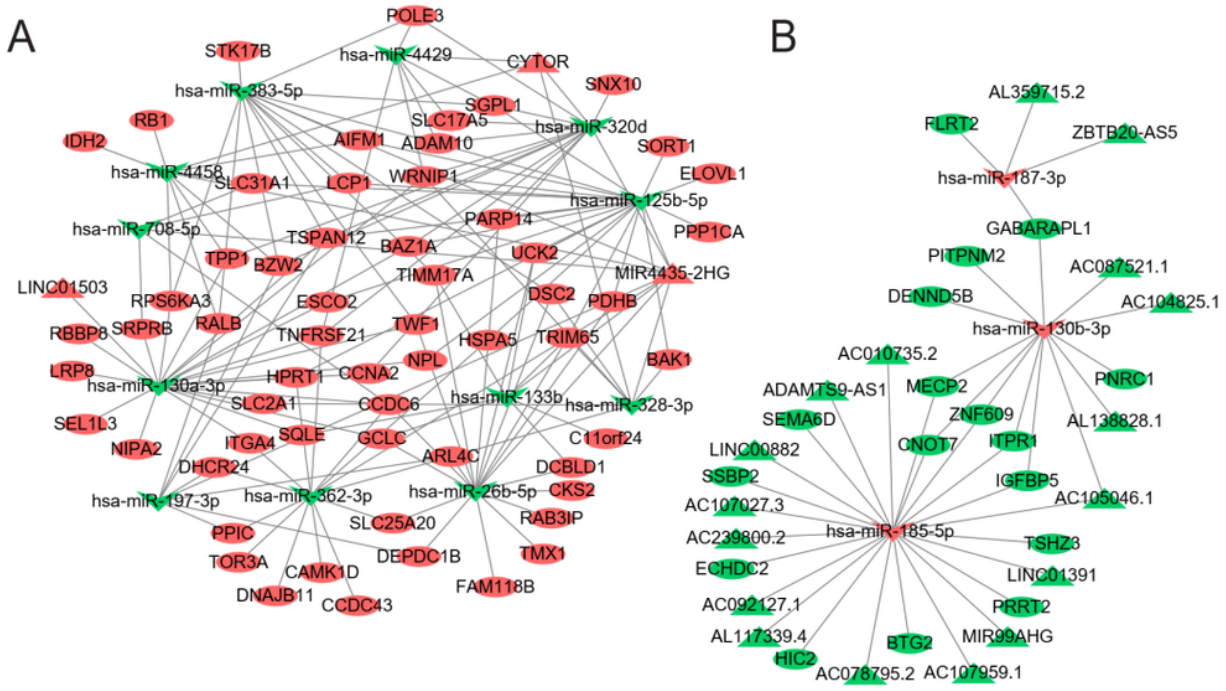

Figure 3

ceRNA networks in EOC patients. (A) up-regulation network. (B) down-regulation network. Ellipses represent mRNA, triangles represent lncRNA, "V" represents miRNA, red indicates up-regulation and green indicates down-regulation.

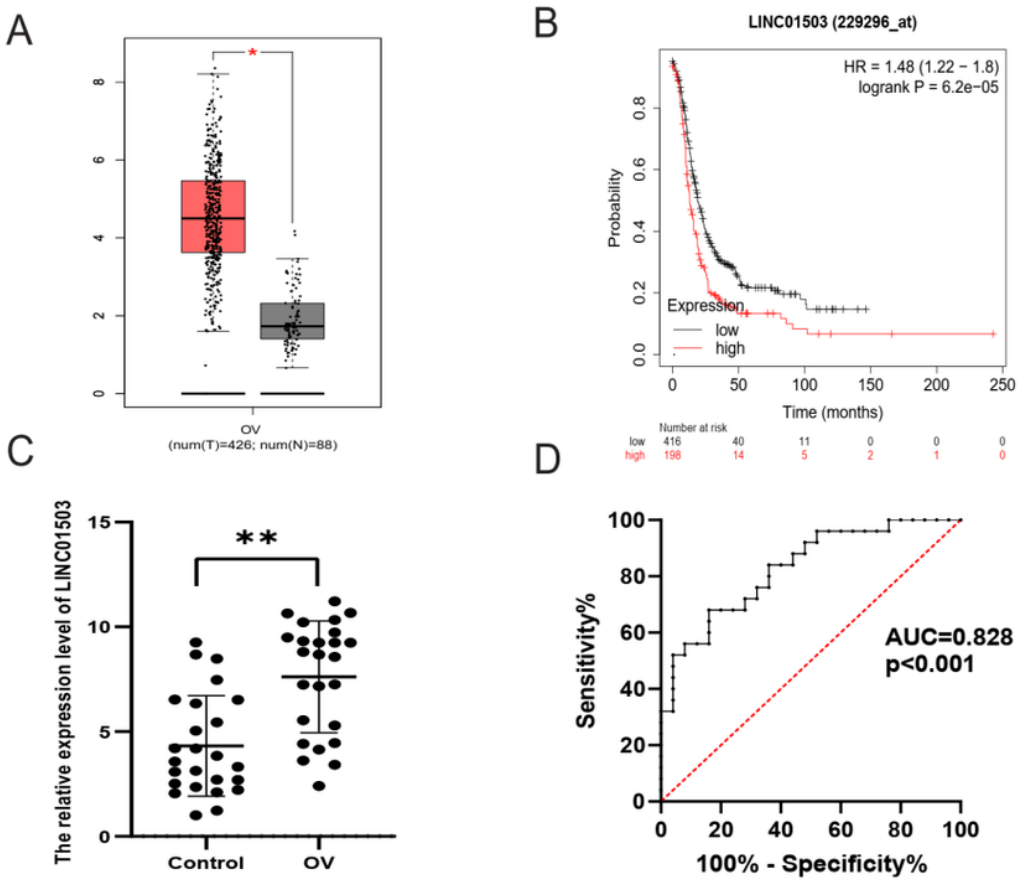

Figure 4

LINC01503 was upregulated in ovarian cancer and related to poor prognosis. (A) Differential levels of LINC01503 in ovarian cancer tissues (n=426) and normal ovary tissues (n=88) analyzed by the TCGA dataset. (B) OS of ovarian cancer patients with high and low expression levels of LINC01503. (C) The expression of LINC01503 in ovarian cancer tissues and adjacent tissues were detected with RT-qPCR. (D) Receiver operating characteristic (ROC) curve analysis of LINC01503 (95% CI: 0.717-0.93, **p <0.01). *P<0.05; **P<0.01.

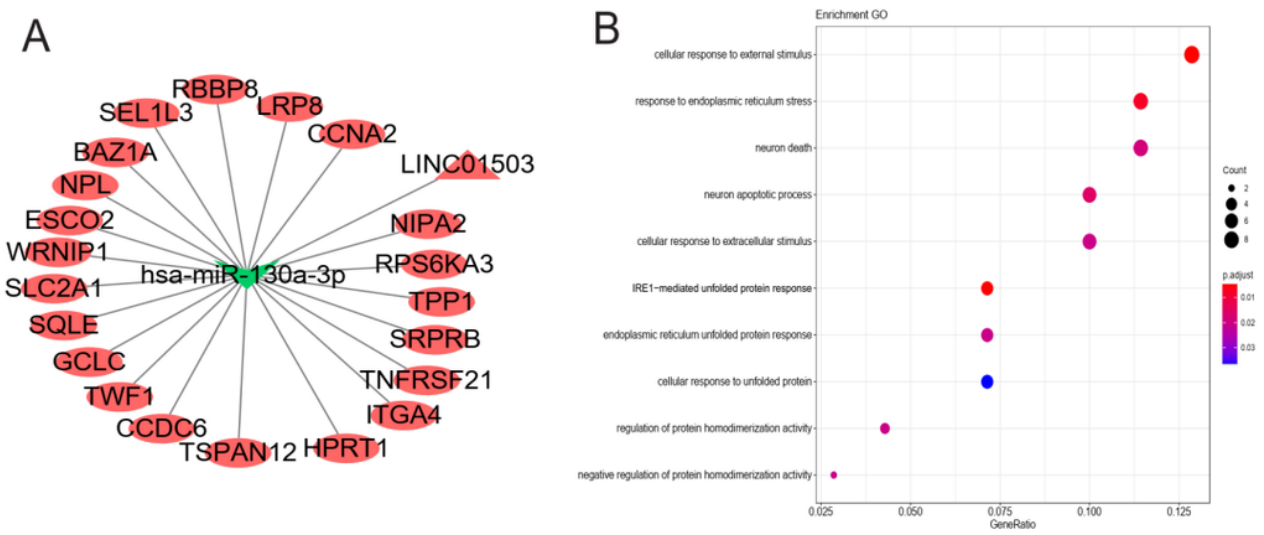

Figure 5

Construction of ceRNA network of LINC01503 and GO enrichment analysis of LINC01503 related mRNAs. (A) lncRNA–miRNA–mRNA network, triangles represent lncRNA, ellipses represent mRNA, "V" represents miRNA, red and green represent up-regulation and down-regulation. (B) Bubble graph of GO enrichment analysis of LINC01503 competitive mRNAs. The color band represents the p-value, and the dots represent the counts of enriched mRNA.

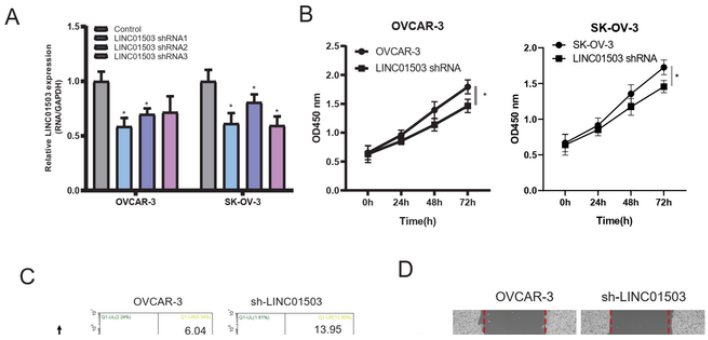

Figure 6

LINCRNA01503 is regulated by CTBP1 in in ovarian cancer. (A) QRT-PCR assay showing the relative LINCRNA01503 expression in OVCAR-3 or SK-OV-3 cells line treated with LINCRNA01503 shRNA1, shRNA2, or shRNA3, respectively. (B) CCK-8 assay showing the proliferation of OVCAR-3 or SK-OV-3 cell line treated with LINCRNA01503 shRNA1. (C) Representative flow cytometry showing that the LINCRNA01503 shRNA1 promote apoptosis of OVCAR-3 or SK-OV-3 cell. (D) Would healing assay showing the migration effect of LINCRNA01503 shRNA1 on OVCAR-3 or SK-OV-3 cell (E) ChIP peaks for CTBP-1 in the SK-OV-3 cell lines. (F) Luciferase reporter assay performed in SK-OV-3 cell lines showing the binding of CTBP-1 and LINCRNA01503. (G) QRT-PCR assay showing the relative CTBP-1 and LINCRNA01503 expression in SK-OV-3 cells line treated with CTBP-1 shRNA. Results are expressed as the Mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001****p < 0.0001 compared with control group.