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CPEB3 Targets E-Cadherin mRNAs in a Post-Transcriptional Regulation Manner and Inhibits the Invasiveness of Ovarian Cancer Cells

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Key words
Ovarian cancer, Bioinformatics, CPEB3, Tumor suppressor gene, E-cadherin

ABSTRACT

CPEB3 (cytoplasmic polyadenylation element binding protein 3) is a key factor that controls poly A tail extension during translation, exhibiting a tumor suppressor effect in several kinds of tumors. However, its expression characteristics and functional role in ovarian cancer (OC) remain unclear. In this study, we aimed to investigate the characteristics and mechanism of CPEB3 in OC. CPEB3 expression data from patient specimens, TCGA and GEO database were evaluated to assess its expression level in OC. Moreover, the effects of CPEB3 on tumor were further evaluated through nude mice xenograft model. We found that mRNA and protein levels of CPEB3 were downregulated in OC. Ectopic expression of CPEB3 decreased proliferation and invasion of OC. GO and KEGG enrichment analysis was performed to investigate the possible network of CPEB3 in ovarian cancer. RNA-binding protein immunoprecipitation confirmed the binding of CPEB3 and E-cadherin. Western blot, and transwell invasion assay confirmed that CPEB3 inhibited the invasion of OC cell by promoting E-cadherin translation. The results implied that CPEB3 plays an important role in the invasion and proliferation of OC and may serve as a target for the development of novel therapeutic strategies.

INTRODUCTION

Ovarian cancer (OC) is a commonly highly invasive tumor in gynecology and one of the major causes of malignancy-related deaths in women with gynecological malignancies (Yeung *et al*., 2015; Webb and Jordan, 2017). In 2020, there were about 310,000 new cases of OC and 210,000 deaths reported worldwide, making it the eighth most common cancer in women (Sung *et al*., 2021). Early-stage OC is mostly asymptomatic, leading to delayed diagnoses and poor prognosis, with a 5-year survival rate of less than 45% (Colombo *et al*., 2006; Elias *et al*., 2018). Currently, the primary therapies for OC are surgery and chemotherapy. Despite substantial improvements to surgical techniques, individualized chemotherapy, and Poly (ADP-ribose) polymerase inhibitors (PARPi) targeted therapy, there remain significant challenges with drug resistance and recurrence (Chandra *et al*., 2019; Keyvani *et al*., 2019). Therefore, clinical and scientific researchers continue to work actively to address these challenges associated with OC.

In eukaryotic cells, mRNAs containing the cytoplasmic polyadenylation element (CPE) are typically dormant mRNAs due to their short 3' poly A tail (20-40 bp), restricting their entry into the translation process. To undergo translation, the 3' poly A tail must be extended to a certain length, which typically occurs during cell enters mitosis or after fertilization, and is regulated by specific proteins (Charlesworth *et al*., 2013). The CPE binding protein (CPEB) is a key regulator of this process, which can inhibit or activate the translation process through binding to the CPE (Mendez and Richter, 2001). CPEB3 is one of CPEB family member and plays a critical role in synaptic plasticity and memory formation in model organisms (Fioriti *et al*., 2015; Qu *et al*., 2020b). Additionally, CPEB also exhibits anti-cancer properties in various tumors,

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including colon cancer, liver cancer, cervical cancer, melanoma through different ways (Hansen *et al*., 2009; Pérez-Guijarro *et al*., 2016; Kang *et al*., 2020).

Several studies have investigated the mechanism of CPEB3 in tumorigenesis and development. It was demonstrated that CPEB3's involvement in the reciprocal interaction between TAMs (tumor-associated macrophages) and CRC (colorectal cancer) cells via modulating IL-6R/STAT3 signaling (Zhong *et al*., 2020). Moreover, it was also found that post-transcriptional regulation of metadherin (MTDH) by CPEB3 suppresses the epithelial-mesenchymal transition and metastasis of hepatocellular carcinoma (HCC), and highlighted the prognostic significance of CPEB3 in HCC tissue (Zhang *et al*., 2020). Additionally, CPEB3 was identified as a promising prognostic marker for melanoma patients with poor survival (Zhang and Liang, 2021). However, its potential effect and role as a tumor suppressor in OC remain unclear.

In this study, OC chip data were analyzed to observe the expression of CPEB3 in OC. Molecular biology and bioinformatics analyses were used to explore the targeted gene network regulated by CPEB3. In addition, the relationship between CPEB3's translation regulation of E-cadherin and its inhibition on OC cell proliferation and invasion was also explored. This study aims to lay a theoretical foundation for exploring new targets for treating OC.

MATERIALS AND METHODS

Data and bioinformatics analysis

Gene expression data of ovarian cancer (OC) was obtained from TCGA portal, the corresponding normal ovarian tissue data was obtained from genotype-tissue expression (GTEx), and differentially expressed genes (DEGs) related to OC was screened. The expression profiles of GSE6008, GSE26712, and GSE66957 were downloaded from GEO database (https://www.ncbi.nlm.nih.gov/geo/) and processed using R package GEO qurey. The boxplots were generated using package ggplot2 in R software. Additionally, CPEB3-RIP sequencing chip of GSE159731 (*Mus musculus*) from GEO database and human HCC sourced from Zhang *et al*. (2020) were integrated to identify genes may interact with CPEB3, the resulted datasets were imported into STRING (https://cn.string-db.org/) for analysis, to obtain the interaction network information. Furthermore, top 100 genes related to OC prognosis were obtained via gene cards (https://www. genecards.org/), and series of genes was cross-compared with the above gene set to obtain overlapping genes.

For functional enrichment analysis, R package org. Hs.eg.db (v3.1.0) was utilized to perform GO/KEGG annotation, cluster profiler (version 3.14.3) was employed for analysis, with minimum gene set of 5 and maximum gene set of 5000. $P < 0.05$ and a false discovery rate (FDR) of < 0.25 were considered significant.

Materials and cell lines

Ovarian cancer tissue chip (number ZL-OVA961) containing 80 samples of OC tissue and 7 samples of normal ovarian tissue was obtained from WellBio technology Co., Ltd. (Table I). Human ovarian cancer cell lines (SK-OV3, A2780, 3AO and CA-OV3), and normal ovarian epithelial cell line (IOSE80), were from Chinese Academy of Sciences. Cells were cultured in RPMI 1640 medium incubated at 37°C under 5% CO_2 with 10% fetal bovine serum. The main materials and reagents are summarized in Supplementary Table SI.

Table I. Clinicopathological features of ovarian cancer tissue chips.

Clinico-pathologic features		Cases	H-score	P value
Age	<50	18	80.38±17.82	0.52
	≥50	62	81.23±14.87	
Clinical stage	I-II	39	81.24±15.39	0.45
	III-IV	41	80.83±15.73	
Pathology	High-grade serous carcinoma	46	78.91±15.21	
	Endometrioid adenocarcinoma	9	81.66±10.04	
	Clear cell carcinoma	9	91.54±6.59	0.04
	Mucinous carcinoma	14	77.87±25.64	
	Low-grade serous carcinoma	2	92.54±3.53	
Peritoneal metastasis	yes	37	79.47±15.63	0.20
	no	43	82.38±15.38	
P53 mutation	yes	67	74.14±19.89	0.03
	no	13	82.37±14.26	
Ki-67	<50	41	84.31±15.79	0.02
	≥50%	39	77.59±14.53	
ER	+	58	79.79±15.67	0.16
	-	22	84.32±14.76	
PR	+	34	79.48±15.3	0.77
	-	46	82.18±15.66	

Lentivirus transfection and siRNA transfection

To generate stable CPEB3-overexpressing cell lines (SK-OV3-CPEB3), as well as negative control (SK-OV3-Control), lentivirus vectors containing GFP label were introduced into SK-OV3 cells (2×10^5 cells/well) at a multiplicity of infection of 20. After one week of culturing in RPMI1640 with 10% FBS, ampicillin and kanamycin containing puromycin (2 μg/mL), the stable SK-OV3 transfectants were obtained. To interfere with the expression of E-cadherin, siRNA particles were transiently transfected into SK-OV3-CPEB3 cells in six-well plate (2×10^5 cells/well) with Lipofectamine 2000.

Immunohistochemical (IHC) analysis

IHC analysis was performed on OC tissue chips to investigate CPEB3, EGFR, BCL2, and E-cadherin proteins expressions. Initially, chips were placed at 60°C for 30 min, treated with xylene I, II, and III for 10 min each, and washing with ethanol gradient (100 to 75%) for 5 min each. Subsequently, the chips were washed utilizing tap water before undergoing antigen retrieval using a citrate buffer (at pH of 6.0). The activity of endogenous peroxidase was subsequently blocked with 3% H_2O_2 for 15 min, washed 3 times with PBS (5 min each). Then incubated with goat serum blocking solution for 1 h at room temperature, primary antibodies (CPEB3, EGFR, BCL2 and E-cadherin) were added and incubated overnight at 4°C. The chips were then incubated with a secondary antibody (goat anti-rabbit) for 30 min at 37°C. Finally, the chips were stained with hematoxylin, then sealed with neutral gum, and observed using a microscope. The results were interpreted by grading staining intensity (with point of 0 for negative coloring, 1 for light yellow, 2 for light brown, and 3 for dark brown), and by assessing positive range (0-25, 26-50, 51-75, and 76-100%) under light microscopy.

CCK-8 cell proliferation assay

SK-OV3 and A2780 cells at logarithmic growth phase that have expressed CPEB3 were collected and adjusted to a density of 2×10^4 cells/mL before inoculating in a 96-well plate. Each group comprises 3 replicates and a blank control group. Following 24, 48 and 72 h of culture, added 10 μL of CCK-8 per well. After one more hour of incubation at 37°C, the absorbance was measured (at 450 nm).

Clonogenic growth assay

SK-OV3 and A2780 cells overexpressing CPEB3 at logarithmic growth phase were resuspended in a single-cell suspension, followed by cell counting. A total of 1000 cells were seeded into each well of 6-well plates for each experimental group and continued incubation for 14 days. After washing with PBS, added 1 mL of 4% paraformaldehyde to each well, cells were fixed for 30 min. Then washed with PBS and stained with 1 mL of crystal violet staining solution for 15 min. After several PBS washes, the cells were dried and photographed.

Transwell assay

Matrigel working solution was prepared at a ratio of 8 μL of serum-free medium to 1 μL Matrigel gel. The working solution was then added to the upper chamber of Transwel for 2 h until the Matrigel gel solidified. Cell concentration was standardized to a density of 5×10^5 cells/mL and 100 μL of cells was seeded into the upper cavity of Transwell. An additional 600 μL of medium containing 20% FBS was added to lower cavity. Following 24 h of incubation, cells in the upper chamber were removed using a medical cotton swab, and cells were fixed in formaldehyde for 30 min. After staining with 0.1% crystal violet for 10 min, the cells were washed with PBS, removed from the chamber, and dried at room temperature, and images were obtained using a microscope (Olympus, Tokyo, Japan) for further analysis.

Transplanted tumor in nude mice

Female nude mice (3-4 weeks old) were from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), maintained under specific pathogen-free conditions, with adaptive feeding for a week, ten nude mice were randomly divided into two equal groups: Control and experimental group. SK-OV3-control and SK-OV3-CPEB3 cells were suspended separately in serum-free medium at 1×10^7 cells/mL. A subcutaneous injection of 100 μL of cell suspension was made into the right axilla of each nude mouse. After 4 weeks, the mice were euthanized and the transplanted tumors were removed. The length (L) and width (W) of each tumor were measured, and the tumor volume (mm^3) was then calculated as: $0.52 \times L \times W^2$.

Western blotting assay

The test cells at logarithmic growth period of SK-OV3, A2780 and CPEB3 groups were lysed using a buffer containing protease and phosphatase inhibitors (Roche, Basel, Switzerland). The lysate was subjected to 10% SDS-PAGE and transferred to a PVDF membrane, then was incubated with the appropriate primary antibodies, followed by immunoblotting with the second antibody. The immunoblot was scanned with Odyssey CLx infrared imaging system (LI-COR, Lincoln, NE, USA).

RNA immunoprecipitation (RIP) assay

The EZ-Magna RIP kit was used to perform RIP

assay. In brief, SK-OV3 cells (1.0×10^7) were lysed using the RIP lysis buffer, then incubation with protein A/G magnetic beads, either IgG or CPEB3 antibody. The RNA was extracted from the complexes formed by the magnetic beads-binding. Subsequently, an agarose PCR assay was used to detect *CDH1* mRNA bound by IgG or CPEB3 antibody.

Statistical methods

All experiments in this study were independently conducted 3 times to ensure reliability, with results presented as mean ± SD. One-way ANOVA analysis was conducted using SPSS 13.0 (P <0.05).

RESULTS

CPEB3 expression is reduced in OC

The expression differences of *CPEB3* gene in OC and normal ovarian tissues were compared. A significant decrease of *CPEB3* expression in OC tissues was observed (Fig. 1A). To validate the findings, three OC chip datasets (GSE6008, GSE26712 and GSE66957) from GEO database were analyzed, and found that *CPEB3* expression was significantly lower in OC compared to normal ovarian tissue, suggesting the role as a tumor suppressor gene in OC (Fig. 1B).

As proteins are the executive units of vital activity, we further examined CPEB3 protein levels in OC and normal ovarian tissues. IHC analysis indicated that 75 of 80 OC cases were positive, with positive rate at 93.6%. In comparison, all 7 cases of normal ovarian tissue revealed positive, indicating a positive rate of 100%. The expression of CPEB3 protein in OC tissues decreased significantly (Fig. 1C).

Subsequently, CPEB3 protein expression were detected in SK-OV3, A2780, 3AO, and CA-OV3, as well as normal ovarian cell lines (IOSE80) using Western blotting. Results demonstrated CPEB3 expression was more or less reduced across all four OC cell lines, especially significantly reduced in SK-OV3 and A2780, so they were selected for subsequent research (Fig. 1D).

Inhibitory role of CPEB3 in OC cell proliferation and invasion

To evaluate the functional role of CPEB3 in OC, we overexpressed CPEB3 in two OC cell lines, SK-OV3 and A2780, to observe changes in OC cell proliferation and invasion (Fig. 2A). CCK-8 assays showed that OC cell proliferation slowed down after overexpression of CPEB3, indicating that CPEB3 plays an inhibitory role in OC cells (Fig. 2B). Similarly, clonogenic growth experiments

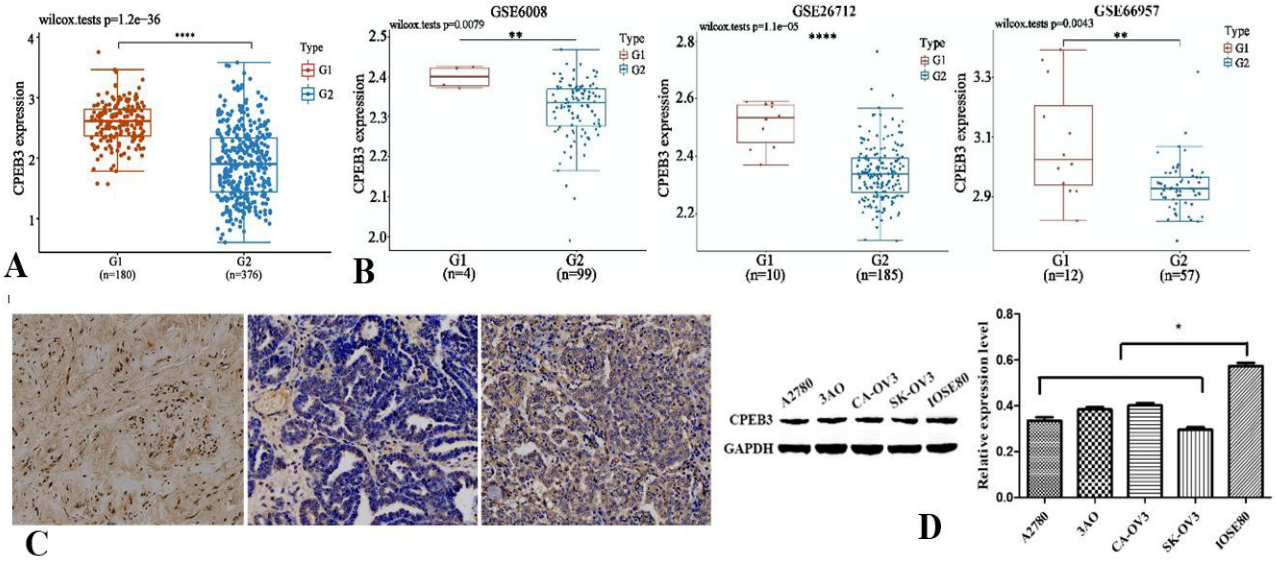

Fig. 1. The expression of CPEB3 is reduced in ovarian cancer.
A, CPEB3 expression in ovarian cancer (OC) tissues (G2) from the TCGA database was significantly lower than that in normal ovarian samples (G1) from GTEx. **B,** CPEB3 expression in OC was significantly lower in three GEO datasets than that in normal ovarian tissue. **C,** Immunohistochemical (IHC) analysis of OC tissue chip showed that 75 OC cases were positive and 5 cases were negatively expressed, resulting in a positive rate of 75/80 (93.6%). In normal ovarian tissue, 7 cases were positive and 0 cases were negative, with a positive rate of 7/7 (100%). **D,** Western blot was used to detect the expression level of CPEB3 protein in various OC cell lines.

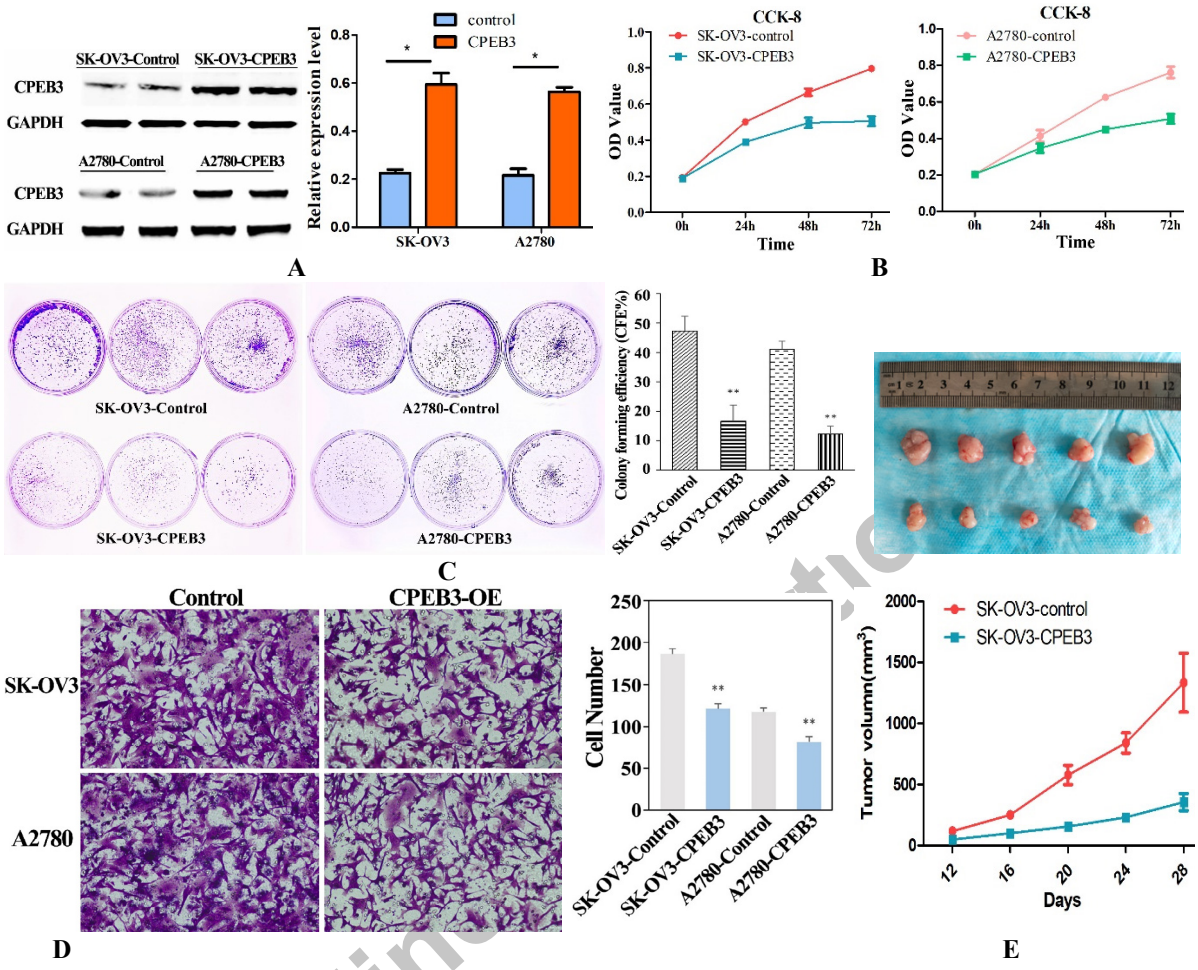

Fig. 2. CPEB3 significantly inhibited the ability of proliferation and invasion of ovarian cancer *in vitro* and *in vivo*.
A, Construction of CPEB3-stabilized OC cell lines using lentiviral vectors in SK-OV3 and A2780 cells. **B,** CPEB3 overexpression resulted in a reduction in OC cell proliferation through a CCK-8 assay. **C,** Clonogenic growth experiments revealed that CPEB3 overexpression significantly reduced the clonal formation ability of OC cells. **D,** In the transwell experiment, the ability of OC cells overexpressing CPEB3 to penetrate cell membranes was significantly weakened. **E,** *In vivo* experiments involving nude mice subcutaneously inoculated with SK-OV3 cells overexpressing CPEB3 showed a significant reduction in tumor volume compared to the control group.

revealed that overexpression of CPEB3 significantly reduced the clonal formation ability of OC cells, thereby inhibiting OC proliferation (Fig. 2C). Moreover, transwell experiment demonstrated that the ability of OC cells to penetrate membranes was significantly weakened by overexpressing CPEB3, suggesting that CPEB3 could inhibit OC's invasion ability (Fig. 2D).

In addition, *in vivo* experiments were carried out, whereby SK-OV3 cells overexpressing CPEB3 were subcutaneously inoculated into nude mice. Results indicated that the tumor volume in the group overexpressing CPEB3 was notably smaller compared to the control group (Fig. 2E), thereby confirming the inhibitory effect of CPEB3 on the proliferation of OC *in vivo*.

Data set analysis of CPEB3 regulatory network in OC

Differential analysis of TCGA and GTEx databases identified 6406 DEGs related to OC compared to normal ovarian tissues (Supplementary Fig. S1A, B). To identify genes directly regulated by CPEB3 in OC, these DEGs were overlapped with the 'CPEB3-RIP' dataset, resulting in a dataset A comprising 424 genes. The 424 genes in dataset A were imported into STRING online analysis, and the interaction network information was derived (Supplementary Fig. S1C). Furthermore, the top 100 genes related to OC prognosis obtained from GeneCards

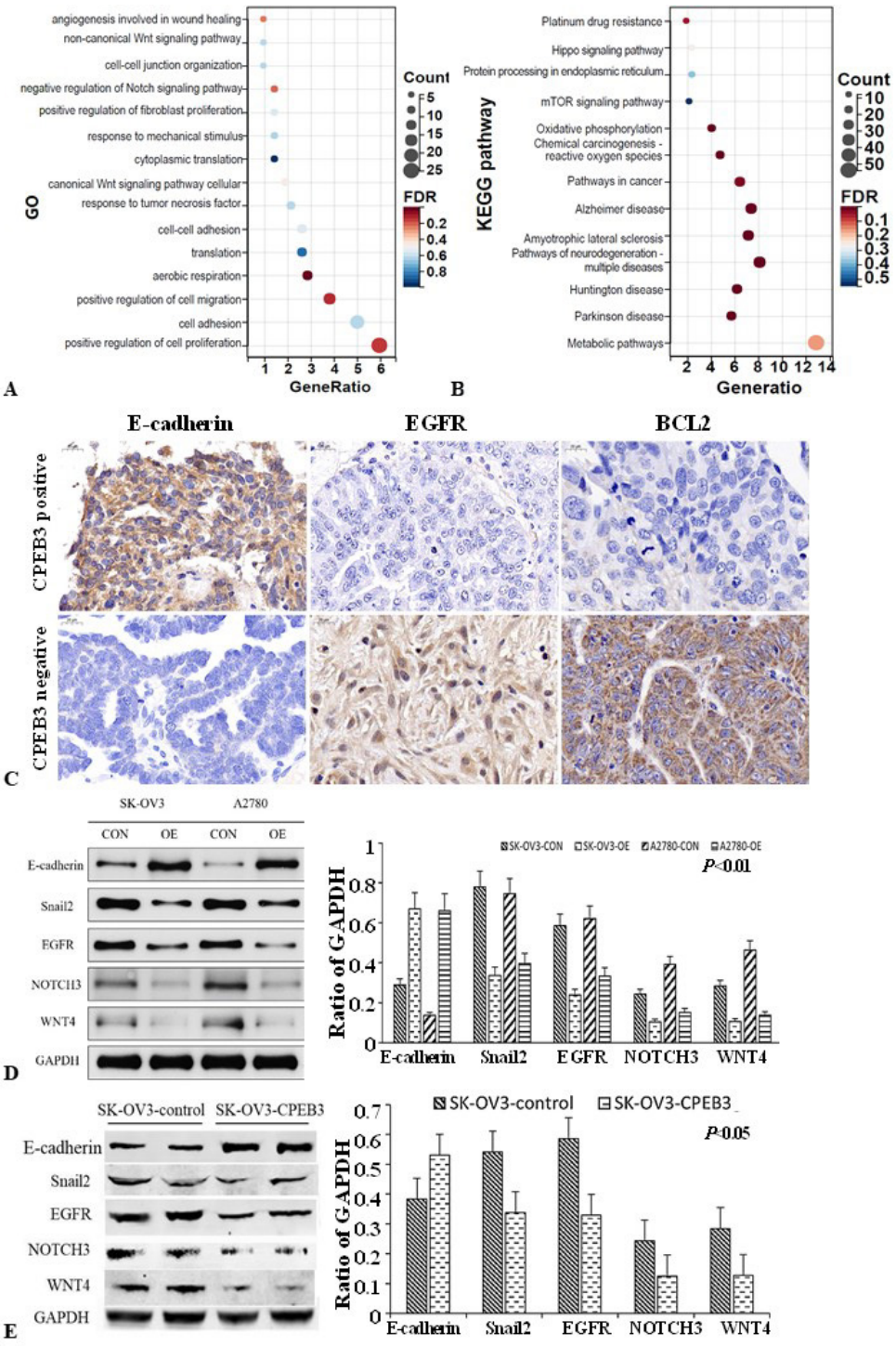

Fig. 3. Functional enrichment analysis and expression analysis of key genes in the CPEB3 regulatory network of ovarian cancer. **A, B,** GO terms and KEGG pathway enriched in differentially expressed CPEB3-realated genes in ovarian cancer. **C,** Immunohistochemical analysis showing the expression of E-cadherin, EGFR and BCL2 in ovarian cancer tissue chips (Bars represent 20 μm). **D,** Western blotting showing the expression of related proteins in SK-OV3 and A2780 cell lines overexpressing CPEB3 (CON: blank control group, and OE: overexpression CPEB3; p <0.01, compared to control group). **E,** Western blotting analysis of related proteins in tumor tissues from nude mice overexpressing SK-OV3-CPEB3 (p <0.05, compared to control group).

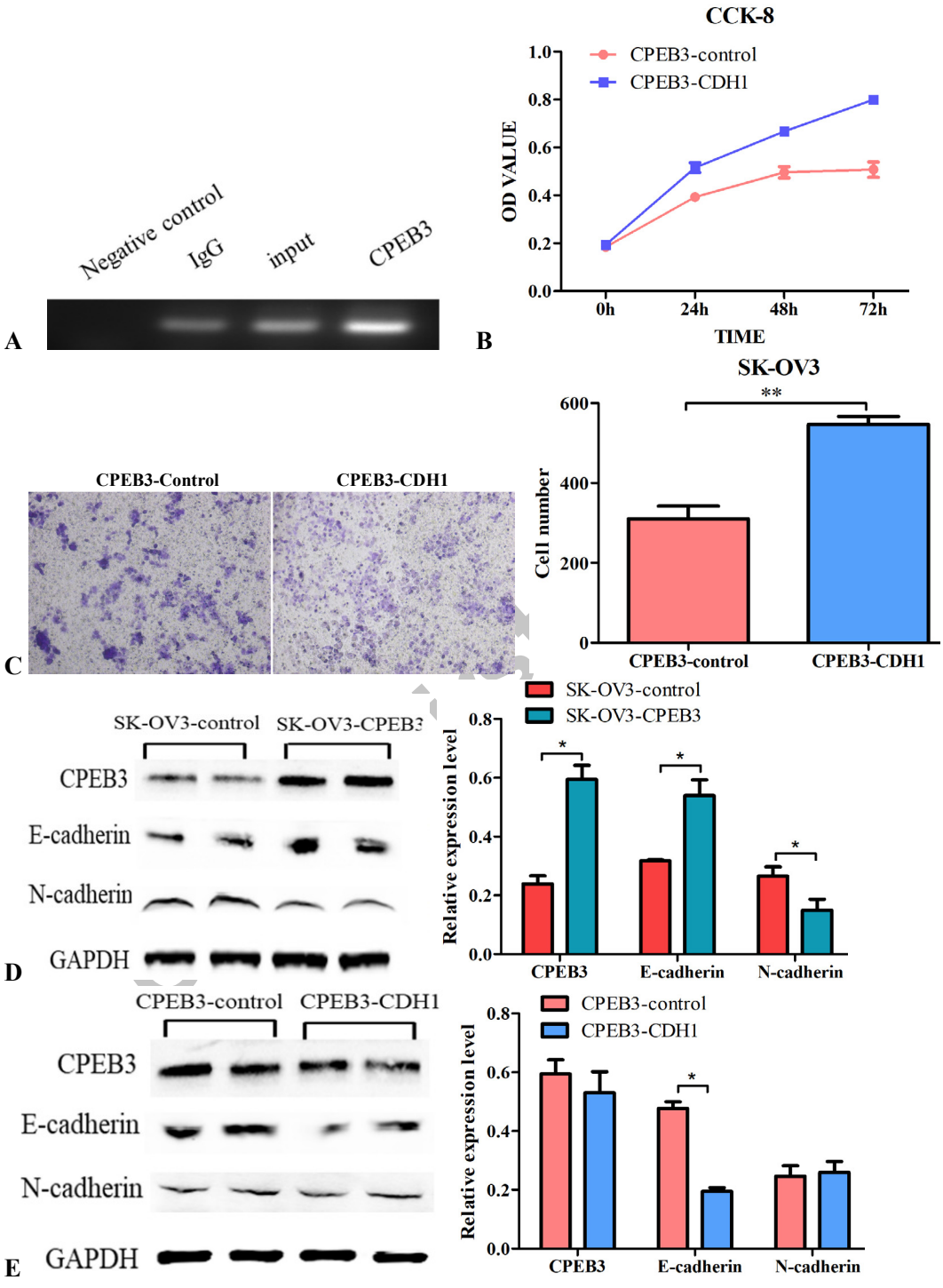

Fig. 4. CPEB3 inhibits invasion by regulating E-cadherin in ovarian cancer.
A, RIP experiment was carried out to verify CPEB3 directly interact with E-cadherin in ovarian cancer cell line SK-OV3. **B, C,** after interfering *CDH1* with siRNA in SK-OV3 ovarian cancer cells expressing CPEB3, cell proliferation and invasion abilities were detected using CCK-8 assay and Transwell assay, respectively. **D,** Western blotting showing the changes in E-cadherin and N-cadherin expression levels after overexpression of *CPEB3* in SK-OV3 cells. **E,** Western blotting displaying E-cadherin and N-cadherin expression levels after interfering *CDH1* with siRNA in SK-OV3 ovarian cancer cells expressing CPEB3.

were cross-compared with the above gene set, leading to the identification of three overlapping genes (*CDH1*, *EGFR*, and *BCL2*) (Supplementary Fig. S1A). The core gene set was mainly enriched in biological processes and molecular functions like cell proliferation, cell adhesion, cell migration, and Wnt and Notch signaling pathways (Fig. 3A). KEGG pathway analysis revealed the core gene set was mostly enriched in tumor-related pathways, including metabolism, oxidative phosphorylation, platinum resistance, and neurological dysfunction diseases (Parkinson's, Huntington's, Alzheimer's diseases), and hippocampal signaling pathway (Fig. 3B).

To confirm the levels of E-cadherin, EGFR, and BCL2 proteins in OC tissues, immunohistochemistry were conducted in OC and normal ovarian tissue chips. The results indicated a lower positivity rate of E-cadherin in OC tissues compared to normal tissues, while positivity rates of EGFR and BCL2 proteins were higher, compared those in normal tissues (p <0.05) (Fig. 3C). Combined with the CPEB3 immunohistochemistry analysis, it was observed that in CPEB3-negative OC tissues, E-cadherin score was lower while EGFR and BCL2 scores were higher, revealing a correlation between CPEB3 and these proteins.

The levels of E-cadherin, snail2, EGFR, NOTCH3, and WNT4 proteins were further detected using Western blotting in SK-OV3 and A2780 cell lines overexpressing CPEB3 gene and in tumor tissues from nude mice overexpressing SK-OV3-CPEB3. A remarkably increase in expression of CDH1 (E-Cadherin) upon overexpression of CPEB3 was observed, whereas the levels of snail2, EGFR, NOTCH3, and WNT4 was notably reduced (Fig. 3D, E).

CPEB3 inhibits proliferation and invasion by regulating E-cadherin in OC

To further investigate whether CPEB3 can inhibit invasion and proliferation in ovarian cancer by regulating E-cadherin, we confirmed that CPEB3 directly regulated E-cadherin in the SK-OV3 ovarian cancer cell line by utilizing the RIP assay (Fig. 4A). Next, we assessed the impact of E-cadherin knockdown on the proliferation and invasion abilities of SK-OV3 ovarian cancer cells with CPEB3 overexpression. The proliferation and invasion of OC were significantly decreased in SK-OV3-CPEB3-control group than SK-OV3-CPEB3-CDH1 group (Fig. 4B, C). Additionally, Western blotting was used to assess the levels of E-cadherin and N-cadherin in SK-OV3 of OC after over-expression of CPEB3 (Fig. 4D, E). The findings indicated that both CPEB3 and E-cadherin expression levels were elevated, whereas N-cadherin expression level was decreased in the SK-OV3-CPEB3 (P <0.05). Furthermore, when compared to control, E-cadherin expression levels were notably reduced in the CPEB3-CDH1 group (P <0.05).

DISCUSSION

Dormant mRNA usually has a poly (A) tail of about 20-40 nucleotides long, which can be extended to 100-150 nucleotides (D'Ambrogio *et al*., 2013). CPEB is a family of sequence-dependent RNA-binding proteins that regulate the translation process induced by mRNA polyadenylation in all eukaryotic cells. It acts by binding to CPE of the 3'-UTR of the target gene mRNA and extends the polyA tail (Mendez and Richter, 2001). CPEB3, one of CPEB family, was first found in the oocytes of Xenopus laevis, causing cells to enter mitosis from the G2-like phase of meiosis (D'Ambrogio *et al*., 2013; Fernandez-Miranda and Mendez, 2012). The main functional region of CPEB protein is the C-terminal region. The C-terminal region is highly conservative (Mendez and Richter, 2001), containing two RNA recognition motifs (RRM) and a Cys6-His2 zinc finger structure (Zif). Among them, the RRM domain is necessary for 3'-terminal CPE of mRNA (co-ordered as UUUUUAU), while the zinc finger structure is a binuclear zinc-binding ZZ-type zinc finger structure. At present, CPEB1 molecules are the most studied molecules in the CPEB family. The binding of CPEB1 molecules to CPE elements can either inhibit translation or activate the translation process (Kim and Richter, 2006; Novoa *et al*., 2010).

CPEB mRNA contains multiple miRNA binding sites, and miRNA is considered to be a key regulator of CPEB function (Morgan *et al*., 2010). At present, the regulation of CPEB3 expression is mainly focused on the study of miRNA. In hepatocellular carcinoma, miR-224 (Miao *et al*., 2020), miR-18a-5p (Cui *et al*., 2021), miR-9-5p (Shu *et al*., 2021) miR-20b-5p (Li *et al*., 2021) were reported to accelerate malignant phenotype by suppressing CPEB3.

Most of the current studies on CPEB3 focus on the key roles of synaptic plasticity and memory (Qu *et al*., 2020a, b). The role of CPEB3 as a tumor suppressor gene in tumors has been gradually reported (Fang *et al*., 2020; Zhang *et al*., 2020; Zhang and Liang, 2021; Zhong *et al*., 2020). However, the mechanism of CPEB3 in tumorigenesis and development has not been elucidated, especially the study of CPEB3 in ovarian cancer is rarely reported. In this study, combined the differential genes of ovarian cancer with CPEB3 binding genes, we have analyzed the GO/KEGG gene enrichment of the genes that CPEB3 may play a role in ovarian cancer.

An epithelial-to-mesenchymal transition (EMT) is reported to be involved in OC (Klymenko *et al*., 2017;

Zhao *et al*., 2020), the down-regulation of E-cadherin, an epithelial marker, is considered to be a sign of the process. In our study, we found that CDH1, which encodes the E-cadherin protein, was showed a significant differential expression. This aroused our interest, so in the following mechanism study, we continued to explore whether the inhibition of ovarian cancer invasion by CPEB3 is related to the inhibition of CDH1 translation by CPEB3. The expression of E-cadherin and its prognostic value in OC tissue microarray was evaluated (Rosso *et al*., 2017). By simulating the spread of ovarian cancer cells under monolayer and unanchored conditions, the cell lines could be divided into mesenchymal cells (M; TOV-112), intermediate mesenchymal cells (IM; SK-OV3), intermediate epithelial cells (IE; OAW-42) and epithelial cells (E; OV-90). Mesenchymal cells have the strongest migration ability in monolayer growth. In this study, the SK-OV3 cell line with partial mesenchymal cells was selected. After overexpression of CPEB3, the invasiveness of cells decreased. After transient transfection of si-E-cadherin in overexpressed CPEB3 cells, the expression of E-cadherin was down-regulated and the invasiveness of cells increased. Combined with the results of CPEB3-RIP experiment, it is suggested that CPEB3 in ovarian cancer may inhibit tumor invasion by regulating the translation of E-cadherin. Further research in this area could lead to the identification of new therapeutic targets for the treatment of ovarian cancer.

CONCLUSION

To sum up, our study provided novel evidence that low expression of CPEB3 may contribute to increased proliferation and invasion of ovarian cancer, possibly through the inhibition of E-cadherin translation by CPEB3. Overall, our study sheds light on the potential role of CPEB3 in the development and progression of ovarian cancer.

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IRB approval and ethical statement

Not applicable.

Ethics statement

The animal study was approved by the Medical Research Ethics Committee of The First Affiliated Hospital of Kunming Medical University (KMMU2020MEC000).

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20210829050829

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

CPEB3 Targets E-Cadherin mRNAs in a Post-Transcriptional Regulation Manner and Inhibits the Invasiveness of Ovarian Cancer Cells

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Supplementary Table SI. Main materials and reagents utilized in the research.

Items	Producers/Sources
Ovarian cancer tissue chip (number ZL-OVA961)	WellBio technology Co., Ltd., Shanghai, China
Human OC cell lines (SK-OV3, A2780, 3AO, and CA-OV3), and normal ovarian epithelial cell line (IOSE80)	Chinese Academy of Sciences, Shanghai, China
10% fetal bovine serum	Gibco, Waltham, MA, USA
CCK-8 assay kit	Lianke Biotech. Co., Ltd., Hangzhou, China
Transwell Matrigel	356234, BD Biosciences, Mississauga, ON, Canada
GAPDH, primary antibodies CPEB3, E-cadherin, EGFR, BCL2, and N-cadherin	Cell Signaling Technology, Beverly, MA, USA
CPEB3 (ab10883) and secondary goat anti-rabbit antibody (ab6721)	Abcam, Cambridge, MA, USA
Green fluorescent protein (GFP)	Gene Chem Co., Ltd., Shanghai, China
siRNA particles	Guangzhou Ruibo Co., Ltd., Guangzhou, China
Lipofectamine 2000	Thermo Fisher Scientific, Waltham, MA, USA
EZ-Magna RIP kit and IgG antibody	Millipore, Billerica, MA, USA
Female nude mice (3-4 weeks old)	Vital River Laboratory Animal Technology Co., Ltd., Beijing, China

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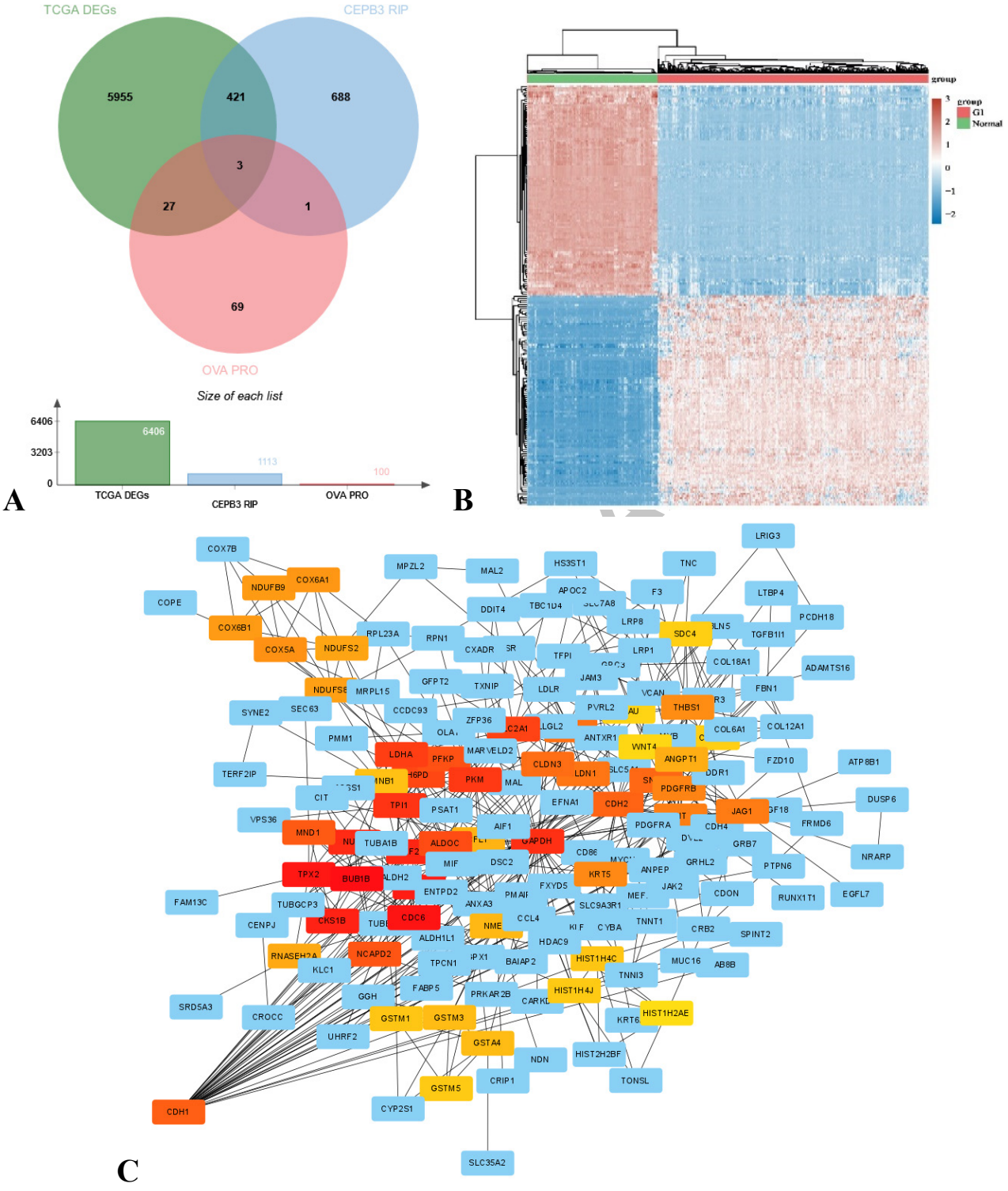
0030-9923/2023/0001-0001 $ 9.00/0



Supplementary Figure S1. Data set analysis of CPEB3 regulatory network in ovarian cancer.
A, Venn diagram illustrating the differentially expressed genes (DEGs) related to *CPEB3* in ovarian cancer. **B,** Heatmaps of DEGs (G1: cancer tissues from TCGA database; Normal: normal ovarian tissue from GTEx database). **C,** Core gene set based on 424 genes were analyzed using cytoHubba in Cytoscape software. Color gradient of nodes indicate the degree of the proteins.