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ORIGINAL ARTICLE

CCT2 prevented β-catenin proteasomal degradation to sustain cancer stem cell traits and promote tumor progression in epithelial ovarian cancer

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Abstract

Background Epithelial ovarian cancer (EOC) is featured by rapid progression and dismal outcomes clinically. Chaperonin Containing TCP1 Subunit 2 (CCT2) was identified as a crucial regulator for tumor progression, however, its exact role in EOC remained largely unknown.

Methods CCT2 expression and prognostic value in EOC samples were assessed according to TCGA dataset. Proliferation and mobility potentials were assessed by CCK8, colony-formation, wound healing, and Transwell assays. Cancer stem cell (CSC) traits were evaluated by RT-PCR, WB assays, sphere-forming assay and chemoresistance analysis. Bioinformatic analysis, co-IP assays and ubiquitin assays were performed to explore the mechanisms of CCT2 on EOC cells.

Results CCT2 highly expressed in EOC tissues and predicted poor prognosis of EOC patients by TCGA analysis. Silencing CCT2 significantly restrained cell proliferation, migration, and invasion. Moreover, CCT2 could effectively trigger epithelial-mesenchymal transition to confer extensive invasion potentials to EOC cells, Importantly, CCT2 positively correlated with CSC markers in EOC, and CCT2 knockdown impaired CSC traits and sensitize EOC cells to conventional chemotherapy regimens. Contrarily, overexpressing CCT2 achieved opposite results. Mechanistically, CCT2 exerted its pro-oncogene function by triggering Wnt/β-catenin signaling. Specifically, CCT2 could recruit HSP105-PP2A complex, a well-established dephosphorylation complex, to β-catenin via direct physical interaction to prevent phosphorylation-induced proteasomal degradation of β-catenin, resulting in intracellular accumulation of active β-catenin and increased signaling activity.

Conclusions CCT2 was a novel promotor for EOC progression and a crucial sustainer for CSC traits mainly by preventing β-catenin degradation. Targeting CCT2 may represent a promising therapeutic strategy for EOC.

Keywords Epithelial ovarian cancer · Chaperonin Containing TCP1 Subunit 2 · Epithelial–mesenchymal transition · Cancer stem cell · Wnt/β-catenin signaling

Introduction

One of the most prevalent and deadly forms of malignant tumors, Epithelial ovarian cancer (EOC), contributes significantly to cancer mortality and morbidity [1]. It is thought that ovarian CSCs' proliferative and differentiating abilities are what cause tumor development, progression, recurrence, metastasis, and therapeutic resistance [2]. Therefore, CSCs may be a useful therapeutic target for treatment of EOC and improve the disease's poor prognosis. At present, several markers for ovarian CSCs have discovered, including epithelial cell adhesion molecule (EpCAM), BIM1, KIT, and NANOG [3, 4]. Besides, it has been demonstrated that signaling pathways modulating normal stem cell development, such as c-myc, transforming growth factor beta (TGF-β), Notch, Hedgehog, and wnt signaling, are linked to the development of cancer and oncogenesis [5, 6]. Given the similarities between CSCs and tissue progenitor cells, it is possible to get insight into CSCs by studying the molecular underpinnings of normal stem cells, making it easier to identify CSC targets for cancer treatment.

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Chaperonin containing TCP1 subunit 2 (CCT2) is a subunit of the CCT chaperonin complex. There are seven other subunits, TCP-1, CCT3, CCT4, CCT5, CCT6a, CCT7 and CCT8 that form the protein folding complex [7, 8]. Recently, it was reported that CCT2 facilitates autophagic clearance of solid protein aggregates independently of ubiquitin and the TRiC complex [9, 10]. Yang et al. revealed that extracellular vesicles (EVs) produced by mesenchymal stem cells (MSCs) reduce liver ischemia/reperfusion injury via regulating the expression of CCT2 [11]. In colorectal cancer, Seong et al. found hypoxia enhanced the interaction of Gil and CCT2 and further activated the hedgehog signaling, which was associated with the survival rate of CRC patients [12]. Interestingly, CCT2 was identified as a key gene for mesenchymal stem cell osteogenic differentiation [13]. However, Whether CCT2 is involved in the regulation of stem cell-like characteristics of tumor cells remains unclear. Although CCT2 was found to be highly expressed in other cancer cells in previous studies, its biological function in epithelial ovarian cancer has not been reported.

In our study, we first analyzed the expression of CCT2 in cancerous and adjacent tumor tissues through TCGA database and analyzed the prognostic value of CCT2 expression in different tumor types. In addition, the effects of CCT2 expression on the growth, invasion, and resistance to chemotherapeutic drugs of epithelial ovarian cancer cells were explored by cell biological function experiments. Our study identified a novel molecular marker associated with stem-like cell characteristics, which provides an important reference value for targeting clear epithelial ovarian cancer stem cells in the future.

Materials and methods

Cell culture

OVCAR3, SKOV3, HEY, A2780 and H08910 cells were acquired from Fudan University, Shanghai Cancer Center. The cell lines were cultured in DMEM medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) and penicillin/streptomycin, and maintained in an incubator at 37 °C containing 5% CO_2.

TCGA analysis

The TCGA-OC cohort's transcriptome RNA-seq data and associated clinical parameters were retrieved from the National Cancer Institute's TCGA database. The cancerous and non-cancerous feature of expression of CCT2 were assessed from GEPIA databases. The K–M plot database (http://kmplot.com/analysis) was used to obtain follow-up information on patients with various cancers. The representative images of EOC tissue and adjacent tumor tissue were acquired from HPA database. Primary tumor samples in the TCGA-OC cohort were assigned a high or low score based on their CCT2 expression quartile. The R package "limma" was used to perform gene expression differentiation analysis, and gene set enrichment analysis (GSEA) was used to further analyze DEGs. Subsequently, pathway enrichment analyses were conducted by the WikiPathways database (wikipathways.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/ or http://www.genome.jp/kegg/). Pathways with p-value < 0.05 were considered statistically significant.

Quantitative real-time polymerase chain reaction

Using the RNA isolation kit (Takara, Dalian, China), total RNAs were extracted from liver cancer cells in accordance with the manufacturer's protocols. In brief, the Prime Script RT Reagent Kit (Takara) was used to reverse-transcribe 600 ng of RNA in a 10 µL reaction volume. Using a Bio-Rad CFX96 thermal cycler (Bio-Rad, Hercules, CA) and a Takara TB Green PCR Kit, a quantitative polymerase chain reaction (qPCR) was performed to detect target genes expression, which was determined using the $2^{-\Delta\Delta Ct}$ method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a normalizing control. The primer used in this study are showed in Supplementary Table 1.

Western blot assay

On ice, proteins were extracted with RIPA buffer (Beyotime, Shanghai, China). Using 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis, equal amounts of protein extracts were separated and transferred onto PVDF membranes (ImmobilonP, Millipore, Darmstadt, Germany). The membranes were blocked for 20 min with Quick-Block Western Solution (Beyotime) before being incubated with the appropriate primary antibodies overnight at 4 °C. The membranes were then incubated for 2 h at room temperature with secondary antibody (Beyotime). Enhanced chemiluminescence was used to detect the protein bands (Beyotime). The antibodies used in this study were as follows: rabbit anti-CCT2 (1:10,000; Abcam, Cambridge, MA), rabbit anti-Ecadherin (1:1000; Cell Signaling Technology, Danvers, MA), rabbit anti-Ncadherin (1:1000; CST), rabbit anti-slug (1:1000; CST), rabbit anti-snail (1:1000; CST), rabbit anti-GAPDH (1:5000; Proteintech), rabbit anti-BIM1 (1:1000; CST), rabbit anti-KIT (1:1000; CST), rabbit anti-β-catenin (1:1000; CST), rabbit anti-HSP105 (1:2000; Abcam), rabbit anti-PP2A (1:500; Abcam), rabbit anti-CCND1 (1:1000; CST), and rabbit anti-myc (1:1000; CST), rabbit anti-TCP1 (1:5000; Abcam), rabbit anti-CCT5 (1:5000; Abcam).

Cell transfection

To establish stable CCT2 overexpressing cell lines, HEY tumor cells were transfected with lentiviral particles. The lentiviruses were generated by co-transfecting HEK293T cells with PCDH-empty vector (Empty vector) or PCDH-CCT2-OE (CCT2-OE) plasmids, along with psPAX2 and pMD2.G helper plasmids. After 72 h of transfection, the viral supernatant was collected and clarified by passing it through a 0.45 μm filter to remove live cells. The concentrated virus was obtained by overnight incubation at 4 °C with a solution containing 25% PEG8000 and 0.75 M NaCl. The collected lentiviruses were then used to infect HEY cells in the presence of polybrene (8 μg/mL), followed by centrifugation at 3000 × g for 20 min to enhance transduction efficiency. After infection, the cells were treated with puromycin (2 μg/mL or 4 μg/mL) for 72 h to select for stable cell lines. The surviving cells were subsequently seeded into 96-well plates to obtain single-cell-derived clones. The overexpression efficiency of CCT2 in the generated single-cell-derived clones was confirmed using quantitative real-time polymerase chain reaction (qRT-PCR) and Immunoblotting.

siRNA, shRNA

For shRNA and siRNA-mediated knockdown assays, Small interfering RNAs (siCon, siRNA targeting CCT2) were purchased from GenePharma Company (Shanghai, China). siRNAs were transfected into SKOV3 cells using Lipofectamine™ 2000 (Invitrogen™), according to manufacturer's protocol. The lentiviral shRNA targets (shCCT2-1 and shCCT2-2) and the negative control (shCon) have been cloned into the pLV3 vector. Lentiviruses were packaged in HEK293T cells according to described above. Cells were infected with diluted lentivirus for 48 h, and then the puromycin-containing medium was added to select the successfully infected cells. The sequences of siRNA or shRNA were listed in Supplementary Table 1.

Sphere formation assay

EOC cells (1×10^3) were cultured in sphere medium containing DMEM/F12 medium (Invitrogen, Carlsbad, CA, USA) supplemented with B27 (Invitrogen), 20 ng/mL EGF (Sigma), 20 ng/mL basic FGF (Invitrogen), and 20 ng/mL HGF (Invitrogen) for two weeks. Under a microscope at 200 magnifications, spheroids were counted and representative fields were photographed at the end of the 2 weeks.

Colony formation

Cells transfected with the indicated plasmids were plated in six well plates at a density of 500 cells per well and cultured for 14 days at 37 °C in a 5% CO_2 atmosphere. Following that, the cell colonies were washed twice with PBS, fixed with methanol for 30 min, and stained for 20 min with 0.5% crystal violet. Under a microscope, the colony numbers were counted.

Cell migration assay

To investigate the effect of CCT2 downregulation on cell migration, the shCCT2-carrying cells were subjected to the wound healing assay. In brief, when the cells reached 90% confluency in plates under standard conditions, the scratched cell layers were made down the center of the well using sterile tips. Cell migration was assessed by measuring the distance between the two boundaries of the cellular area at 0 and 48 h respectively.

Cell invasion assay

To assess the effect of CCT2 expression on cell invasion capacity, cell invasion assays were performed using Matrigel-coated chambers (pore size 8 μm; Corning Costar Corporation, Cambridge, MA). Briefly, 5×10^4 cells were placed in an upper chamber coated with Matrigel and supplemented with serum-free medium. The lower chamber was filled with medium containing 10% FBS. The cells were incubated for 48 h at 37 °C, fixed with 4% paraformaldehyde, stained with 0.5% crystal violet and finally counted by inverted microscopy.

Resistance analysis

Cells were counted and seeded at 1×10^4 cells per well in 96-well plates, and cultured for 12 to 24 h. The same dosage of 400 μg/mL 5-flurouracil or 24 μg/mL cisplatin (MCE) was added to the scramble and shCCT2 EOC cells, respectively. The measurement of cell proliferation was performed via the Cell Counting Kit-8 Kit according to the manufacture's protocol (Beyotime, Shanghai, China). Three wells of each group were detected every day and incubated for 2 h with the Cell Counting Kit-8 solution. The absorbance of the sample taken from each well was measured on a microplate reader (Bio-Rad) at 450 nm. The results were plotted as the mean ± SD from three separate experiments with three replicates per experiment for each experimental condition.

Immunoprecipitation

The indicated plasmids were co-transfected into HEK293T cells. After 72 h, cells were lysed with IP lysis buffer (20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, and 1% NP-40) containing protease inhibitor cocktail (Roche, USA). Immunoprecipitation assays were performed

following the manufacturer's instructions (Pierce, Thermo, USA). The supernatant protein was immunoprecipitated with the indicated tagged antibodies and protein G agarose beads overnight at 4 °C. Antibodies used in these experiments are listed below. The complex was washed with NaCl buffer and boiled with 1 × SDS loading buffer.

Ubiquitination assay

SKOV3 cells were co-transfected with the indicated plasmids. The cells were given 10 μM MG132 treatment for 6 h after being incubated for 24 h. The cells were then boiled for 10 min at 100 °C while being lysed with IP lysis buffer. For IP using anti-β-catenin, the supernatants were collected, and RIPA lysis was then added to a cocktail of PMSF and protease inhibitors.

Statistical analysis

GraphPad Prism 8.0 (GraphPad Software, CA) was used for the statistical analyses. The significance of differences between groups was evaluated using the Student t test and one-way analysis of variance. The results from three independent experiments are presented as the mean ± standard deviation. Values of $P < 0.05$ were considered to indicate statistically significant differences.

Results

High expression of CCT2 was associated with poor prognosis of cancer patients

To investigate the clinical significance of CCT2 in EOC, we first obtained CCT2 expression of EOC tissues and normal tissues from the HPA database. The results showed that the intensity and density of CCT2 expression were higher in EOC compared to normal tissues (Fig. 1A). HPA analysis also revealed that CCT2 was highly expressed in epithelial ovarian cancer tissues (Fig. 1A). Using follow-up data from the K–M database, we found that high CCT2 expression was positively associated with poorer overall survival (OS) and progression-free survival (PFS) in patients (Fig. 1B). Moreover, high CCT2 expression in other tumors was also associated with shorter overall survival in patients, including hepatocellular carcinoma (HCC), Adrenocortical carcinoma (ACC), Breast Cancer (BRCA), Head and Neck squamous cell carcinoma (HNSC), Kidney renal clear cell carcinoma (KICH), Lung squamous cell carcinoma (LUAD), malignant mesothelioma (MESO), ovarian tumor (OV) and uterine Corpus Endometrioid Carcinoma (UCEC) (Supplementary Fig. 1).

CCT2 suppressed liver cancer cell proliferation and invasion

We first detected the expression of CCT2 in EOC cell lines (OVCAR3, SKOV3, HEY, A2780, and H08910). The results, obtained by quantitative PCR (qPCR) and immunoblotting, showed that CCT2 expression and protein levels are higher in SKOV3 cells and lower in HEY cells, compared with other cells (Fig. 1C). Next, we successfully silenced the CCT2 expression in SKOV3 cells and overexpressed CCT2 in HEY cells, which were confirmed by RT-PCR and WB assays (Fig. 1D, E). To observe the effects of CCT2 expression on cell proliferation, CCK8 and clone formation assays were performed. The results showed that CCT2 knockdown significantly suppressed cell growth, while CCT2 overexpression enhanced the number of cell growth (Fig. 2A, B). When silencing CCT2 expression in SKOV3 cells, we found the migrative and invasive cells were significant reduced by wound healing assays and Transwell assays. However, overexpressed CCT2 in HEY cells observed the opposite results (Fig. 2C, D). Moreover, WB assays showed that E-cadherin (an epithelial markers) was increased by knockdown of CCT2, while N-cadherin and vimentin (mesenchymal markers) were decreased, as well as EMT-related transcription factor (including snail and slug). While CCT2 expression had an reduced E-cadherin expression and an enhanced mesenchymal markers expression (Fig. 2E). Immunofluorescence analysis found that downregulation of CCT2 decreased vimentin expression but increased E-cadherin expression in SKOV3 cells, while overexpression of CCT2 in HEY cells produced the opposite effects (Fig. 2F, G).

CCT2 enhances the stemness and the chemoresistance of EOC cells

To characterize the effects of CCT2 expression on cancer stem cell, we firstly detected CCT2 expression in sphere cells and non-sphere cells, respectively. RT-PCR results showed that CCT2 and ovarian cancer stemness-related markers, BMI1 and KIT were upregulated in sphere cells, compared to the non-sphere cells (Fig. 3A). Consistently, western blotting results presented that increased stemness-related makers and CCT2 expression in sphere cells (Fig. 3B). TCGA analysis also showed that CCT2 expression was positively associated with the stemness-related markers, including BIM1, KIT and NANOG (Fig. 3C). Additionally, KEGG and WiKipathway analysis showed that genes within wnt signaling (stemness-related pathway) were the only enriched in CCT2 high expression group (Fig. 3D). Expectedly, CCT2-silencing cells formed lesser and smaller spheroids than the control cells, while CCT2-expressing cells had a higher number of spheroids (Fig. 3E). WB assays further revealed that silenced CCT2

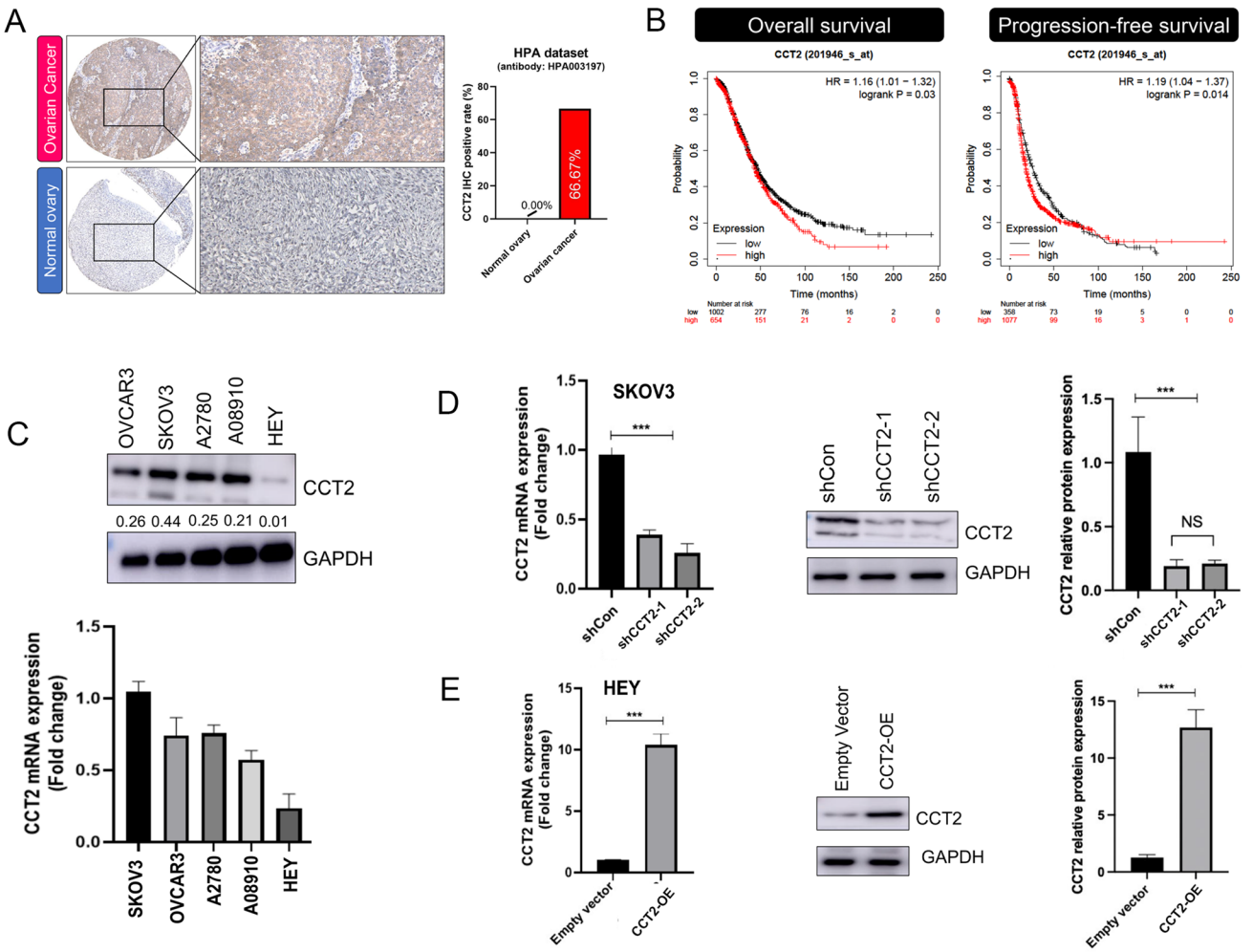


Fig. 1 CCT2 was preferentially expressed in EOC tissues and predicted poor prognosis. **A** Representative images of immunohistology staining of CCT2 in EOC (upper) and normal ovary tissues (lower) according to Human Protein Atlas (HPA) dataset. **B** Kaplan–Meier analysis of overall survival for EOC patients with distinct CCT2 expression states in entire cohort (left). Kaplan–Meier analysis of progression-free survival for EOC patients with distinct CCT2 expression states in entire cohort (right). **C** Expression level of CCT2 in indicated EOC cell lines were assessed by RT-PCR and WB assays. **D** Efficiencies of CCT2 knockdown (upper) and overexpression (lower) in indicated EOC cells were determined by RT-PCR and WB assays. For shRNA-mediated knockdown assay, shCon was used as a negative control. For CCT2 overexpression assay, Empty vector was used as a negative control

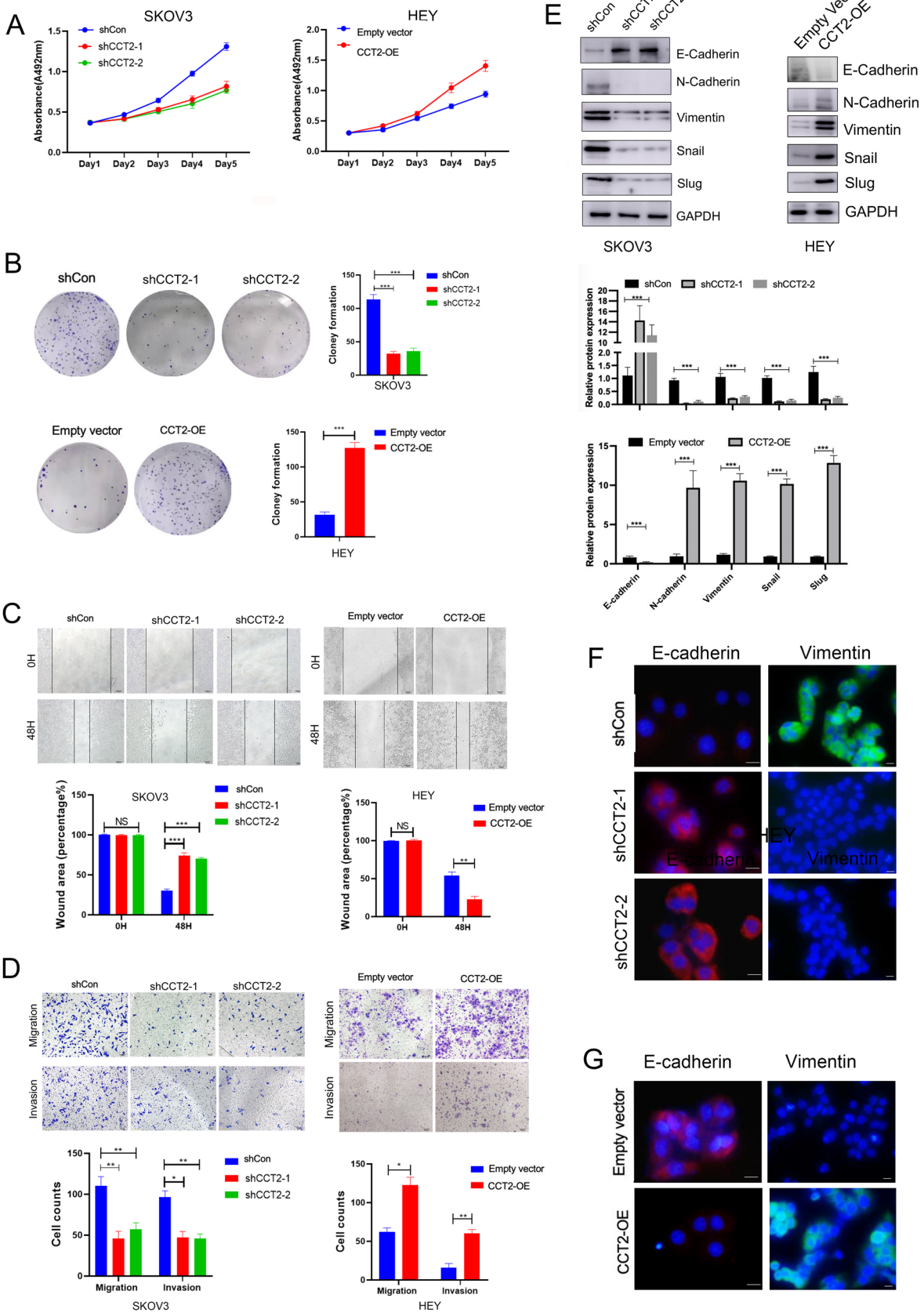
reduced stemness-related markers and overexpressed CCT2 increased stemness-related markers (Fig. 3F).

One typical stemness-related characteristic is resistance to chemotherapeutic drugs.

Cell viability of CCT2-overexpressing cells was considerably higher than that of controls following treatment with two routinely used chemotherapeutic drugs, cisplatin and 5-flurouracil, at various doses. However, CCT2-silencing had the opposite impact on EOC cells' chemoresistance (Fig. 3G). According to these findings, CCT2 knockdown markedly improved the drug sensitivity of EOC to 5-flurouracil and cisplatin.

Identification of Wnt/β-catenin signaling as the downstream crucial target of CCT2

Wnt signaling play critical roles in proliferation and self-renewal of CSCs. To further explore the underlying mechanism of CCT2-regulated progression in EOC, we performed gene set enrichment analysis (GSEA). Interestingly, Wnt/β-Catenin signaling, a well-recognized pathway for regulating tumor progression and CSC traits in EOC, was positively associated with CCT2 in EOC, evidenced by that KEGG_WNT-SIGNALING, WP-WNT_SIGNALING, PID_WNT_SIGNALING PATHWAY, and PID_BETA-CATENIN_NUC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SKOV3
shCon
shCCT2-1
shCCT2-2
Absorbance(A492nm)
Day1 Day2 Day3 Day4 Day5
HEY
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shCCT2-1
shCCT2-2
Cloney formation
SKOV3
Empty vector
CCT2-OE
HEY
C
0H
48H
Wound area (percentage%)
NS
D
Migration
Invasion
Cell counts
E
E-Cadherin
N-Cadherin
Vimentin
Snail
Slug
GAPDH
Relative protein expression
F
E-cadherin
Vimentin
G

◂**Fig. 2** CCT2 promoted malignant potentials and induced epithelial–mesenchymal transition in EOC. **A** CCK8 assays were performed to evaluate the effects of CCT2 expression knockdown (left) and overexpression (right) on the proliferation potentials in EOC cells. **B** Colony formation assays were performed to evaluate the effects of CCT2 expression knockdown (upper) and overexpression (lower) on the proliferation potentials in EOC cells. **C** Wound healing assays were conducted to assess the effects of CCT2 expression knockdown (left) and overexpression (right) on the migration potentials in EOC cells. **D** Transwell assays were conducted to assess the effects of CCT2 expression knockdown (left) and overexpression (right) on the migration/invasion potentials in EOC cells. **E** Effects of CCT2 knockdown (left) and overexpression (right) on the EMT-related markers in indicated EOC cells. **F** Immunofluorescence staining for indicated EMT-related markers after CCT2 knockdown in EOC cells. **G** Immunofluorescence staining for indicated EMT-related markers after CCT2 overexpression in EOC cells

PATHWAY were significantly enriched in CCT2-high subgroups according to TCGA dataset (Fig. 4A). Importantly, applying ICG-001, a specific antagonist targeting Wnt/β-Catenin signaling, could effectively abolish the promotional effects of CCT2 overexpression on the proliferation of EOC cells according to colony formation assays (Fig. 4B). Moreover, ICG-001 treatment could also abolish the increased CSC traits caused by CCT2 overexpression (Fig. 4C). Similarly, enhanced migration and invasion capacities which were attributed to CCT2 overexpression were also attenuated by ICG-001 treatment (Fig. 4D). Of note, inhibition of Wnt/β-Catenin signaling dramatically resensitized CCT2-overexperssed EOC cells to Cisplatin and 5-FU therapy, which exerted extensive resistance after CCT2 overexpression (Fig. 4E, F). Together, these results demonstrated CCT2 mainly exerted its pro-tumoral functions by activating Wnt/β-Catenin signaling in EOC, and inhibiting Wnt/β-Catenin signaling could effectively antagonizing CCT2 overexpression.

CCT2 recruited HSP105-PP2A complex to β-Catenin to prevent phosphorylation-induced degradation

RT-PCR assay demonstrated that the mRNA expression of β-Catenin remained unaltered after CCT2 knockdown (Fig. 5A), suggesting CCT2 might shed influence on the β-Catenin expression at post-transcriptional stage. Interestingly, CHX chasing assays revealed dramatically decreased protein stability due to CCT2 knockdown (Fig. 5B). Moreover, decreased β-Catenin expression could be effectively rescued by applying MG132, a proteasome inhibitor. However, β-Catenin expression could only be little restored by BA, a lysosomal inhibitor (Fig. 5C). Thus, these data further demonstrated that CCT2 was essential for preventing β-Catenin degradation by the proteasome pathway. We next set out to clarify how CCT2 promoted protein stability of β-Catenin. Surprisingly, according to String and BioGRID database, we occasionally found that CCT2 was essential for the interaction between PPP2CA and HSP105 (Fig. 5D). As previous study identified HSP105-PPP2CA complex was critical for the intracellular β-Catenin abundance via dephosphorylation of β-Catenin at N-terminal, and there existed the binding between β-Catenin and CCT2, we raised the hypothesis that CCT2 was the core element to recruit HSP105-PPP2CA complex to β-Catenin to reduce phosphorylated β-Catenin caused by GSK3β. Hence, we first examine the interaction among β-Catenin, CCT2, HSP105 and PPP2CA. co-IP assays indicated that these four proteins were interacted with each other (Fig. 5E). Knocking down CCT2 resulted in reduction of interactions between β-Catenin and HSP105-PPP2CA complex (Fig. 5F). Supportively, CO-IP assays further confirmed that only CCT2 protein was immunoprecipitated by using β-catenin antibody but not other CCT subunits, such as TCP1 and CCT5 (Fig. 5G). More importantly, only CCT2 knockdown reduced β-catenin expression and significantly decreased the number of spheroids. While silencing of TCP1 and CCT5 had no effect on β-catenin expression (Supplementary Fig. 2). Accordingly, active β-Catenin (defined as non-phosphorylated β-Catenin) as well as the total β-Catenin expression was drastically decreased in CCT2-knockdown EOC cells, accompanied with reduction of classical β-Catenin downstream targets such as CCND1 and MYC (Fig. 5H). Finally, ubiquitination assays demonstrated that silencing CCT2 caused greatly increased ubiquitinated β-Catenin (Fig. 5I). Collectively, our data indicated that CCT2 could recruit HSP105-PPP2CA complex to prevent β-Catenin degradation, thus resulting in enhanced CSC traits and progressive phenotypes.

Discussion

Rapid progression of EOC during treatment was a major neck bottle for improving prognosis concurrently. The main contributor for this dismal situation was identified as distinguished invasion capacity of EOC cells, whose driven power largely remained unknown. Here, we reported that CCT2 was a novel regulator for EOC progression, especially in mediating aggressive invasion capacity via promoting EMT process. Moreover, CCT2 contributed to cancer stem cell traits in EOC to induce drug resistance. Importantly, we revealed that CCT2 was essential for the protein stability of β-Catenin by preventing it from ubiquitination-induced protein degradation, thus resulting in mesenchymal phenotype and enhanced cancer stem cell potentials.

In our study, we found that even knockdown of the proteins TCP1 and CCT5, which are functionally related to the TRIC/CCT complex, still failed to attenuate the malignant phenotype of EOC cells. However, knockdown of CCT2 alone significantly prevented tumor cell growth, invasion, and drug resistance. This suggests that CCT2

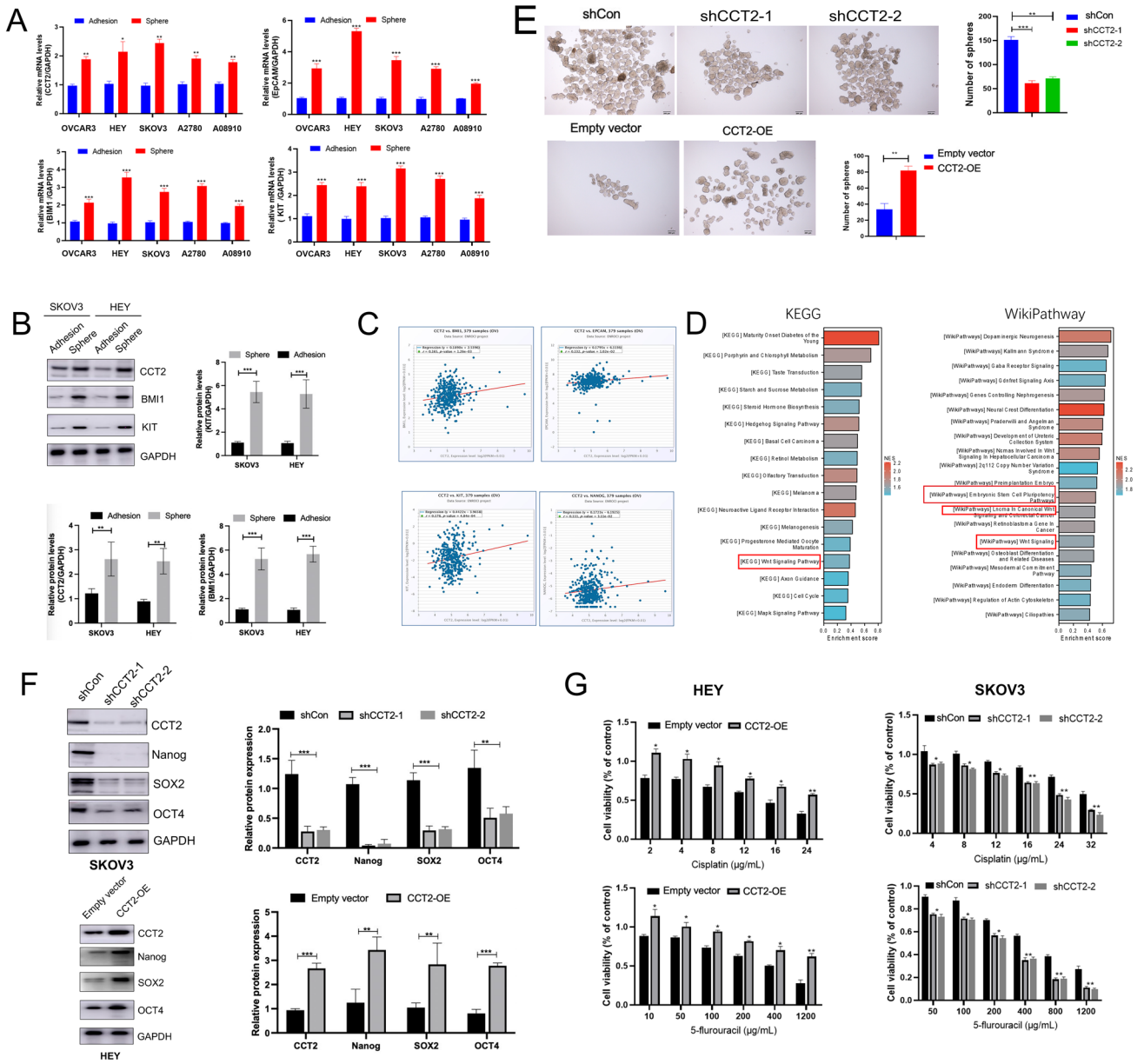

Fig. 3 CCT2 sustained cancer stem cell traits of EOC cells. **A** Expression level of CCT2, BMI1, and KIT in indicated EOC cells and corresponding spheres according to RT-PCR assays. **B** Expression level of indicated proteins in indicated EOC cells and corresponding spheres according to WB assays. **C** Correlations between CCT2 and indicated CSC-related markers according to TCGA ovarian cancer dataset. **D** KEGG and Wikipathway analysis demonstrated pluripotential was positively enriched in CCT2-high group according to TCGA ovarian cancer dataset. **E** Effects of CCT2 knockdown (upper) and overexpression (lower) on the CSC traits according to sphere-forming assays. **F** Effects of CCT2 knockdown (upper) and overexpression (lower) on the CSC-related transcriptional factors according to WB assays. **G** Effects of CCT2 knockdown (upper) and overexpression (lower) on the sensitivity towards indicated drugs according to CCK8 assays

monomers are necessary for promoting EOC progression. Therefore, future intervention of CCT2 is an important modality for OC therapy. Notably, CCT2 is preferentially expressed in EOC tissues, thus downregulation of CCT2 via transducing specific knockout system based on siRNA or antisense oligonucleotide might favor the prognosis in EOC patients with high CCT2 expression and had potential in preventing EOC recurrence and metastasis. Additionally, TCGA analysis revealed that CCT2 was highly expressed in ovarian cancer tissues, which was an indicator of overall survival and progression-free survival in OC patients. This suggests that CCT2 expression may be used for prognostic assessment of OC patients.

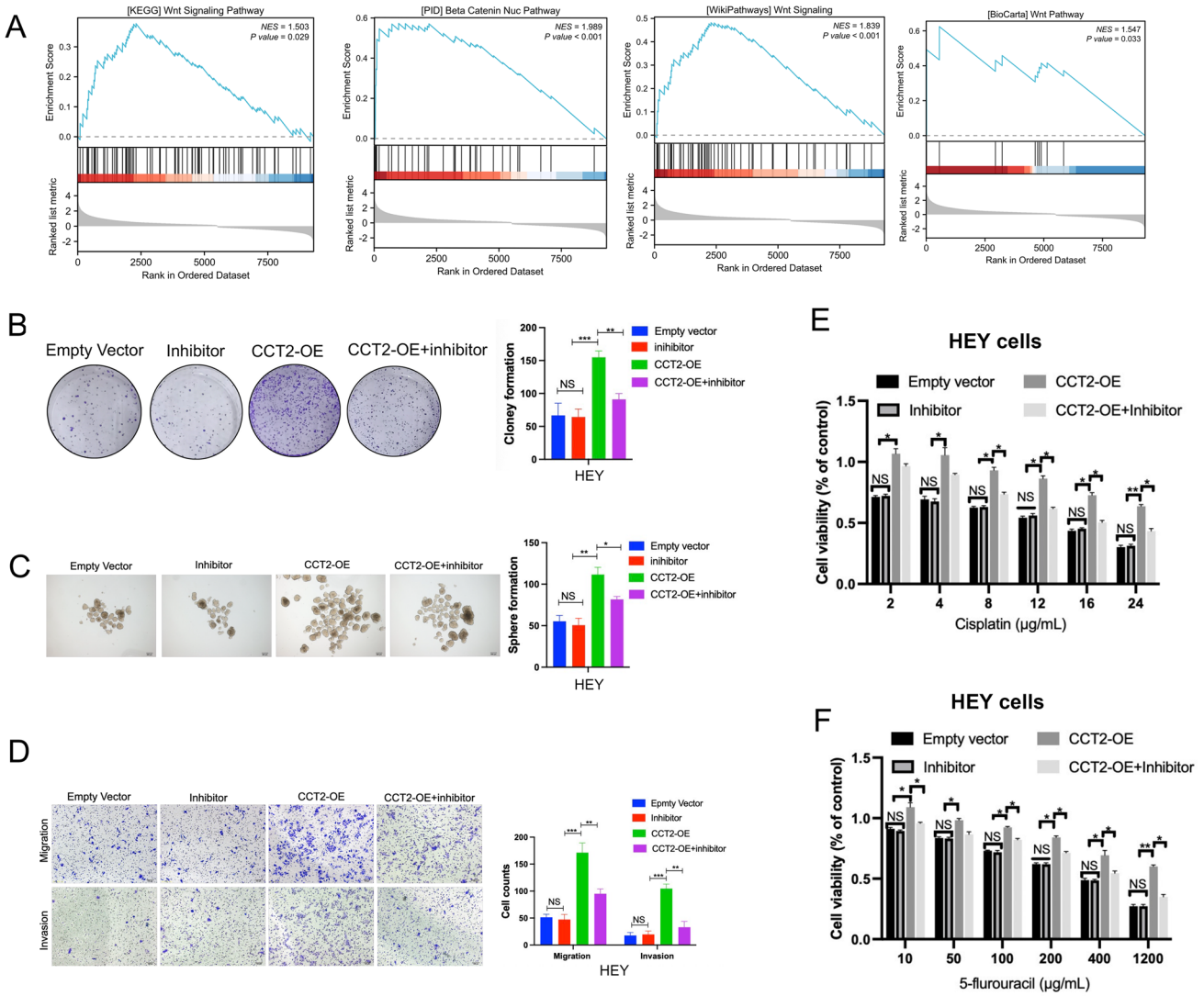

Fig. 4 CCT2 relied on Wnt/β-Catenin signaling to promote invasiveness and CSC traits in EOC. **A** GSEA results indicated significant enrichment of Wnt/β-Catenin signaling activation in CCT2-high group according to TCGA ovarian dataset. **B** Proliferation potentials of EOC cells received indicated treatment were determined by colony formation assays. **C** CSC traits of EOC cells received indicated treatment were determined by sphere-forming assays. **D** Migration and invasion potentials of EOC cells received indicated treatment were determined by Transwell assays. **E** Resistance towards Cisplatin in EOC cells received indicated treatments was assessed by CCK8 assays. **F** Resistance towards 5-FU in EOC cells received indicated treatments was assessed by CCK8 assays

EMT is a crucial and complicated molecular process involved in EOC progression. Previous studies reported that formation of the mesenchymal-like phenotype was essential for the invasiveness and metastasis of EOC, thus identifying EMT as a therapeutic target for preventing EOC progression [14, 15]. In recent years, although several genes have been reported to trigger EMT process, most of which were difficult for inventing novel targetable strategy [16]. Moreover, disrupting altered signaling that regulated EMT process such as HGF/c-Met, PI3K/AKT, or MAPK/ERK also shed nonnegligible adverse effects during treatment, thus laying the emphasis on the searching for novel target for reversing EMT process in EOC [17–19]. Here, we reported that CCT2 as a critical promoter for mediating EMT, and interfering CCT2 expression could almost abolished this process. Our results provided novel insight into the detailed mechanism of EMT formation in EOC.

Metastasis and recurrence were considered as the major contributor for the dismal outcomes of EOC patients [20]. Accumulating evidence revealed that one of such culprits for EOC recurrence and metastasis was the existence of cancer stem cells (CSCs), which were a small fraction of cancer cells possessing robust stem cell capacities, such as unlimited proliferation and differentiation potentials, within tumoral loci [21, 22]. Therefore, CSCs was considered to play vital roles in tumor heterogeneity constitution,

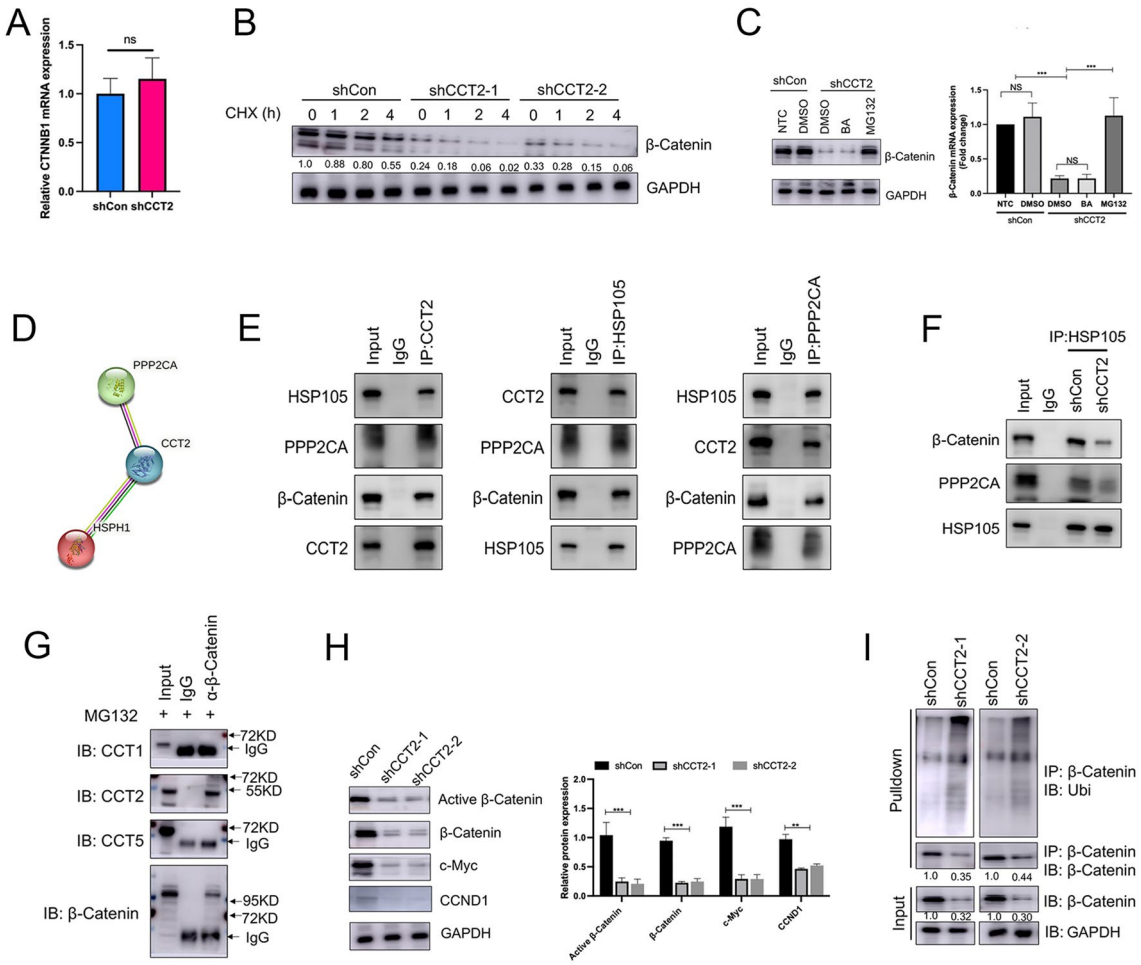

Fig. 5 CCT2 promoted interaction between HSP105-PP2A complex and β-Catenin to prevent phosphorylation-induced proteasomal degradation. **A** mRNA expression of β-Catenin before and after CCT2 knockdown was determined by RT-PCR assays. **B** CHX chasing assays were performed to determine the turnover time of β-Catenin after indicated treatment. **C** Recovery states of β-Catenin expression after receiving indicated treatments was determined by WB assays. **D** Core role of CCT2 in promoting HSP105 binding to PPP2CA, a critical subunit of PP2A, was indicated in STRING database. **E** Co-IP assays confirmed that CCT2, HSP105, PPP2CA, and β-Catenin was physically interacted with each other. **F** Effects of CCT2 knockdown on the interaction between HSP105-PP2A complex and β-Catenin were assessed by co-IP assays. **G** CO-IP assays confirmed the exclusive interaction of β-Catenin with CCT2 but not other CCT subunits. **H** Effects of CCT2 knockdown on the expression of active β-Catenin, total β-Catenin, CCND1, and MYC were determined by WB assays. **I** Ubiquitination assays were performed to assess the effects of CCT2 knockdown on the ubiquitination levels of β-Catenin in EOC

tumorigenesis, tumor relapse, metastasis, and more importantly, the resistance towards conventional anti-cancer regimens. Of note, CSCs was also considered as the potential and ideal target [23, 24]. However, limited understanding of how these CSCs maintain their stemness features was available currently. Most of investigations focused on the membrane marker for identifying CSC subpopulations in EOC, which exerted limited therapeutic benefit in recent years, even in the era of gene-editing [25, 26]. Therefore, looking back to the stemness-associated regulatory signaling pathways which contribute to sustain stem cell potentials might achieve more translational benefit. In present study, we reported that CCT2 was essential for the CSC traits in EOC, evidenced by dramatically decreased sphere-forming capacity. Notably, interfering CCT2 expression could effectively result in sensitizing EOC towards conventional therapy. Our results elucidated a novel intracellular regulator which was crucial for the CSC traits in EOC and provided a potential target for boosting current chemotherapy efficiency, which might have opportunity for improving current poor prognosis in EOC patients with advanced tumor stage.

The Wingless/It (Wnt)/β-catenin signaling was crucial for the normal liver development, metabolic zonation, and regeneration [27, 28]. Aberrant dysregulations of Wnt/β-catenin pathway was closely associated with the alterations of liver microenvironment such as pro-inflammation and immunosuppression microenvironment, and promote initiation, development, and progression of EOC [29, 30].

Specifically, activation of Wnt/β-catenin signaling was reported to excessively involved in EOC recurrence and metastasis [31]. Wnt/β-catenin signaling could directly promote invasiveness-related gene expression and shed important regulatory utility in mediating EMT formation and invasion of EOC cells [32]. Thus, Wnt/β-catenin pathway was identified as the potential key signaling for controlling the drastic invasiveness of EOC cells. Unfortunately, there was no satisfactory therapeutic approach for Wnt/β-catenin signaling concurrently. Although several EOC tumors exerted mutations in genes encoding key effector molecules of Wnt/β-catenin pathway, the percentage was minor, indicating the disadvantage of the strategy of targeting mutation [33, 34]. Hence, striving for a complete understanding of how Wnt/β-catenin pathway was pathologically altered was important for inventing novel therapeutic approach to restrain EOC recurrence and metastasis. Here, we discovered that CCT2 was essential for the protein stability of β-catenin, which was achieved by promoting the formation of the complex of HSP105 and β-catenin, thus paving the way for the subsequent dephosphorylation of β-catenin. This process could effectively antagonize the suppressive effects of active GSKβ-induce β-catenin degradation. Our discoveries pointed out another way for hindering β-catenin activity in EOC, which might facilitate future anti-β-catenin regimen development.

There were several limitations of our present study. First, the prognostic value of CCT2 in Chinese population was lack due to the difficulty in enrolling enough number of EOC patients in our center. However, it should be noted that the prognosis value of CCT2 was clearly demonstrated in TCGA dataset. Second, detailed interaction information between CCT2 and HSP105, CCT2 and β-catenin was not provided in present study, which is undergoing in our lab currently. Finally, the efficiency of targeting CCT2 for restraining CSC traits and enhancing therapeutic effect of conventional chemotherapy was only preliminarily provided, a novel anti-CCT2 approach based on AAV technology is under investigation in our center, whose data might be a useful complementary for present study.

Conclusions

In summary, we identify CCT2 as novel promotor for EMT process and a crucial sustainer for CSC traits in EOC. Moreover, CCT2 was essential for the interaction between HSP105 and β-catenin, thus resulting in dephosphorylation of β-catenin by the PP2A complex. Our data demonstrated the significance of CCT2 in EOC progression and provide novel explanation for the aberrant activation of β-catenin signaling in EOC. Notably, targeting CCT2 might be a promising strategy for preventing EOC recurrence and metastasis.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Raw data are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have declared that no competing interest exists.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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