

CPSF4 Promotes CACNA2D2-Regulated Ca⁺ Channel to Maintain Olaparib Resistance in Stem Cell Selective-Cultured Ovarian Cancer Cells

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Research Article

Keywords: CPSF4, CACNA2D2, olaparib resistance, calcium signaling

Posted Date: May 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9740889/v1>

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Additional Declarations: No competing interests reported.

Abstract

Unlike other types of cancers, direct implantation metastasis is a major cause of death in ovarian cancer, whereas stemness-related spheroid formation is a critical step for cancer cell colonization to the mesothelium. Despite extensive research, directly investigating ovarian cancer stemness under stem-cell-selective culture conditions remains uncommon. Resistance to the PARP inhibitor olaparib remains a major clinical challenge and is closely linked to cancer stemness. In this study, we found that Cleavage and Polyadenylation Specificity Factor 4 (CPSF4), a key regulator of pre-mRNA processing, is significantly upregulated in three-dimensional (3D)-cultured ovarian cancer spheroids and closely associated with cancer stemness. The role of CPSF4 in ovarian cancer remains unclear. Here, we demonstrate that CPSF4 contributes to drug resistance in 3D-cultured ovarian cancer cells. Mechanistically, CPSF4 upregulates CACNA2D2, a key modulator of calcium channel activity, thereby maintaining intracellular calcium homeostasis and promoting stemness-associated adaptation to therapeutic stress. Notably, CPSF4 depletion enhances sensitivity to olaparib. Loss of CPSF4/CACNA2D2 significantly sensitizes ovarian cancer cells to olaparib treatment. Collectively, these findings identify a novel CPSF4–CACNA2D2 axis in ovarian cancer and suggest a potential strategy for overcoming reduced sensitivity to olaparib.

Introduction

Ovarian cancer ranks as the eighth most common cancer and cause of cancer-related death among women globally. In 2022, more than 300,000 people worldwide were diagnosed with ovarian cancer, resulting in over 200,000 deaths. In 2025, in the United States alone, more than 20,000 women are expected to be diagnosed with ovarian cancer, with over 10,000 deaths anticipated (Caruso, Weroha et al. 2025). High-grade serous ovarian carcinoma (HGSOC) is the most common type of epithelial ovarian cancer with the highest mortality. Its poor long-term prognosis is mainly attributed to characteristics such as high metastatic potential and resistance to anticancer drugs (Devenport, Tran et al. 2025, Sun, Wang et al. 2025). A key step in ovarian cancer metastasis involves the detachment of tumor cells from the primary site, their transport through the peritoneal fluid, and subsequent attachment and colonization on the mesothelial lining of organs within the peritoneal cavity, which represents a distinctive and common route of metastasis for this cancer (Bayraktar, Chen et al. 2024, Xu, Verhagen et al. 2024). Elucidating the key signaling pathways underlying this unique metastatic process may offer novel therapeutic avenues for patients.

Recent studies have highlighted a close interplay among cancer stemness, spheroid-forming capacity, and metastatic potential in ovarian cancer (Phadte, Bishnu et al. 2024, Kudo, Greer et al. 2025). Cancer stem cells (CSCs), characterized by their abilities for self-renewal and differentiation, are believed to drive tumor initiation, PARPi resistance, and recurrence (Cordon-Cardo 2010, Nassar and Blanpain 2016). Under anchorage-independent conditions, ovarian cancer cells with enhanced stemness can survive and proliferate by forming multicellular spheroids, which serve as an in vitro surrogate of stem-like properties. These spheroids not only exhibit increased expression of stemness-associated markers, but

also demonstrate strong resistance to anoikis, enabling them to persist in suspension within the peritoneal cavity. Upon attachment to the mesothelial lining of peritoneal organs, these stem-like spheroid cells can re-establish proliferative colonies, thereby promoting peritoneal dissemination(Motohara, Yoshida et al. 2021, Bharti, Dey et al. 2024). Notably, resistance to the PARP inhibitor olaparib, a cornerstone of maintenance therapy in ovarian cancer, has been closely linked to stem-like properties. However, the molecular mechanisms connecting stemness and olaparib resistance remain incompletely understood.

Cleavage and polyadenylation specificity factor 4 (CPSF4) is a core component of the mRNA 3'-end processing machinery, playing an essential role in pre-mRNA cleavage, polyadenylation, and transcript maturation(Schönemann, Kühn et al. 2014, Hamilton and Tong 2020, Liu and Manley 2024). Beyond its canonical function in RNA processing, CPSF4 has increasingly been recognized as an important regulator of gene expression programs that influence tumorigenesis. Recent studies have linked CPSF4 to aberrant cell proliferation, cell-cycle dysregulation, and therapy resistance in several malignancies, including lung cancer, hepatocellular carcinoma, and colorectal cancer(Lee, Zheng et al. 2021, Zhong, Shen et al. 2024). Despite these emerging insights, little is known about the functional role of CPSF4 in ovarian cancer, particularly in high-grade serous ovarian carcinoma (HGSOC), where metastatic dissemination predominantly occurs through a unique detachment–survival–reattachment process. Addressing this gap may not only advance our understanding of the molecular mechanisms driving HGSOC progression but also identify CPSF4 as a promising therapeutic target for limiting tumor spread.

Calcium (Ca^{2+}) signaling is a fundamental regulator of numerous cellular processes. These functions are tightly controlled by diverse calcium channels located on the plasma membrane and intracellular organelles(Berridge, Lipp et al. 2000, Chen, Chen et al. 2013). CACNA2D2, which encodes the $\alpha_{2\delta 2}$ subunit, is essential for proper channel assembly, trafficking, and gating kinetics, thereby shaping intracellular Ca^{2+} dynamics(Gao, Sekido et al. 2000, Hoppa, Lana et al. 2012). Emerging evidence suggests that its function may be highly cancer-type–specific and influenced by microenvironmental cues(Geyer, Ritter et al. 2025). In several tumor types, loss of CACNA2D2 can disrupt calcium channel assembly or function, leading to aberrant Ca^{2+} signaling that promotes uncontrolled cell growth(Carboni, Gao et al. 2003, Buchanan and McCloskey 2016). Conversely, emerging studies indicate that CACNA2D2 may support tumor progression under certain conditions by enhancing calcium influx, facilitating survival signaling, or modulating tumor–microenvironment interactions(Warnier, Roudbaraki et al. 2015). Despite the recognized importance of Ca^{2+} signaling in sustaining cancer stemness, spheroid formation, and resistance to anoikis—processes that are particularly relevant to ovarian cancer progression—the role of CACNA2D2 in these malignant phenotypes remains largely unexplored. Understanding whether CACNA2D2 contributes to Ca^{2+} -dependent stemness regulation and transcoelomic metastasis may therefore reveal previously unrecognized therapeutic vulnerabilities in ovarian cancer.

In this study, we investigated the critical role of CPSF4 in maintaining stemness and promotes stemness-associated resistance to olaparib. Mechanistically, CPSF4 upregulated CACNA2D2, thereby maintaining intracellular calcium homeostasis and facilitating adaptation to olaparib-induced stress.

Collectively, targeting the CPSF4–CACNA2D2 axis significantly enhances olaparib sensitivity. These findings reveal a novel mechanism underlying olaparib resistance and highlight CPSF4 as a potential therapeutic target in ovarian cancer.

Methods and materials

Chemicals and reagents

Olaparib (cat. no. HY-10162R) was purchased from MedChemExpress (Shanghai, China) and prepared as a 10 mM stock solution in dimethyl sulfoxide (DMSO), which was stored at -20°C. Primary antibody against CPSF4 (cat. no. 15023-1-AP) was purchased from proteintech (Wuhan China). Primary antibody against CACNA2D2 (cat. no. sc-365911) was purchased from Santa Cruz Biotechnology (Dallas, USA). Primary antibody against α -Tubulin (cat. no. 66031-1-Ig) was purchased from Proteintech (Wuhan, China). Dulbecco's modified Eagle medium (DMEM), RPMI-1640 medium and fetal bovine serum (FBS) were obtained from Gibco, Thermo Fisher (Waltham, USA).

Cell lines and cell culture

Human ovarian cancer cell lines SKOV3, HEYA8 and the human mesothelial cell line MeT5A were obtained from the American Type Culture Collection (ATCC, USA). Normal ovarian surface epithelial cell lines HOSE and IOSE80 were used as non-malignant controls. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) or RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37°C in a humidified incubator with 5% CO₂.

RNA interference and gene knockdown

The SKOV3 and HEYA8 cell lines with stable CPSF4 knockdown and non-specific control were generously provided by Prof. Wei Guo from Dalian Medical University, Dalian, Liaoning, China. Briefly, short hairpin RNA (shRNA) targeting CPSF4 and the corresponding non-specific control shRNA (NS shRNA) were constructed and transfected into ovarian cancer cells using Lipofectamine 3000 (Invitrogen, USA) according to the manufacturer's instructions. Stable knockdown cell lines were generated by puromycin selection. Knockdown efficiency was verified by quantitative real-time PCR (qRT-PCR) and Western blot analysis.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, USA). Complementary DNA (cDNA) was synthesized using a reverse transcription kit (Takara, Japan). Quantitative PCR was performed using SYBR Green Master Mix (Applied Biosystems, USA) on a real-time PCR system. Relative gene expression levels were calculated using the 2^{- $\Delta\Delta$ Ct} method, with β -actin serving as an internal control. The primers

used were: CPSF4 forward, 5'-TCCAGCAAAGCAGAGAACCC-3'; CPSF4 reverse, 5'-TGCATCTGTTGGCGTAGTGT-3'; CACNA2D2 forward, 5'-ACAA-GGACAATCGGAACCTG-3'; CACNA2D2 reverse, 5'-TGGGCTTTCT-GGAAATTCTCT-3'; β -actin forward, 5'-CATCCTCACCTGAAGT-ACCC-3'; β -actin reverse, 5'-AGCCTGGATAGCAACGTACATG-3'.

Western blot analysis

Cells were harvested and lysed in RIPA buffer containing 50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1 mM PMSF, 1 μ M pepstatin, 0.5 mg/mL leupeptin, and 0.3 μ M aprotinin. Protein concentrations were determined using a BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein (20–30 μ g per lane) were separated by 10–12% SDS–PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% non-fat milk and incubated with primary antibodies against CPSF4, CACNA2D2, or α -Tubulin overnight at 4°C, followed by incubation with HRP-conjugated secondary antibodies of same species for 1 hour at room temperature (RT). Following three washes with TBST buffer (20 min per wash), protein bands were visualized using an enhanced chemiluminescence (ECL) detection system. Bands densities were analyzed and quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Tumor spheroids formation assay

SKOV3 and HEYA8 cells were cultured and spheroid formation was conducted following previously reported methods. Briefly, cells were seeded in ultra-low attachment plates at a density of 5,000 cells/mL in a total volume of 15 mL and maintained in serum-free stem cell culture medium. After 5–7 days incubated at 37°C in a humidified atmosphere containing 5% CO₂, tumor spheroids larger than 50 μ m in diameter were captured and counted under a microscope. The number of spheroids was used to evaluate the self-renewal capacity of cancer stem-like cells. For each experimental group, spheroids were counted in at least three randomly selected microscopic fields, and all experiments were performed independently in triplicate.

Spheroids viability assay

A total of 7.5×10^4 cells were maintained in 15 mL of culture medium. After 3 days, cells were treated with varying concentrations of the indicated drugs for the specified duration. Following treatment, cell pellets were collected into 1.5 mL tubes and incubated with 500 μ L of medium containing 10% MTT solution (5 mg/mL) at 37°C for 4 hours. Subsequently, 150 μ L of DMSO was added to dissolve the formazan crystals completely, and the samples were gently mixed to ensure uniform solubilization. Then, 50 μ L of the solution was transferred to a 96-well plate. Cell proliferation was then measured by recording absorbance at 490 nm using a microplate reader. All experiments were performed in triplicate, and the results were expressed as the mean $\pm$ standard deviation (SD).

Mesothelial adhesion assay

MeT5A mesothelial cells were seeded in 24-well plates to form a confluent monolayer. Ovarian cancer cells were then added onto the monolayer and incubated for corresponding time points under standard culture conditions. After incubation, non-adherent cells were gently washed away with PBS three times to minimize nonspecific attachment. The remaining adherent cells were subsequently fixed with 4% paraformaldehyde and quantified under an inverted microscope. Images were captured from randomly selected fields, and the number of adherent cells was quantified to evaluate the mesothelial adhesion ability of ovarian cancer cells. All experiments were independently repeated at least three times.

RNA sequencing and bioinformatics analysis

Total RNA was isolated from CPSF4-knockdown and control ovarian cancer cells and subjected to RNA sequencing (RNA-seq). Libraries were prepared using a standard protocol and sequenced on an Illumina sequencing platform. Differentially expressed genes (DEGs) were identified using the criteria $|\log_2 \text{fold change}| > 1$ and $P < 0.05$. A Venn diagram was used to identify overlapping DEGs between different cell lines. Functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, was performed to explore biological processes and signaling pathways regulated by CPSF4.

Single-cell RNA sequencing analysis

Single-cell RNA sequencing (scRNA-seq) data were retrieved from the scDVA database (<http://ov.cancer-pku.cn/>), based on a previously published ovarian cancer dataset (Zheng et al., 2023). The expression profile of CPSF4 across different cell populations was analyzed using the online platform. Data normalization and cell-type annotation were performed according to the original study.

Correlation analysis of gene expression

The correlation between CPSF4 and CACNA2D2 expression was analyzed using Spearman correlation analysis in ovarian cancer datasets and normal ovarian tissue datasets. Correlation coefficients and statistical significance were calculated using standard statistical methods.

Ovarian cancer tissue microarray

The human ovarian cancer tissue microarray used in this study was obtained from Shanghai Outdo Biotech Co., Ltd. (Shanghai, China). The microarray comprised 59 paraffin-embedded ovarian cancer specimens and 12 paired adjacent non-malignant ovarian tissue samples collected from patients who underwent surgical resection. None of the patients included had received any form of anticancer therapy, including chemotherapy, radiotherapy, targeted therapy, or immunotherapy, prior to surgical resection. Clinicopathological information, including age, histological subtype, tumor grade, and clinical stage, was provided by the manufacturer when available. For statistical analyses, 2 cases of ovarian cancer tissues and adjacent non-malignant tissues with severe tissue detachment were excluded.

Immunohistochemistry (IHC)

Paraffin-embedded ovarian cancer tissue samples were sectioned and subjected to immunohistochemical staining using antibodies against CPSF4 and CACNA2D2. Briefly, tissue sections were deparaffinized in xylene and rehydrated through a graded ethanol series, followed by antigen retrieval in citrate buffer. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min at room temperature. Sections were incubated with primary antibodies overnight at 4°C, followed by incubation with secondary antibodies and visualization using a DAB detection system. Finally, the stained sections were dehydrated, mounted, and observed under a light microscope. Staining intensity and percentage of positive cells were evaluated to generate IHC scores.

Xenograft tumor model

Animal experiments were conducted in accordance with institutional guidelines. Female BALB/c nude mice (4–6 weeks old) were subcutaneously injected with SKOV3 cells with either CPSF4 knockdown or control cells. Tumor growth was monitored by measuring tumor length and width using calipers every 3–4 days. Tumor volume was calculated using the formula:

$$V = \frac{1}{2} \times length \times width^2$$

At the end of the experiment, tumors were excised and subjected to immunohistochemical analysis.

Statistical analysis

All experiments were performed at least three independent times. Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using the latest version of GraphPad Prism software (San Diego, CA, USA). Differences between groups were evaluated using Student's t-test or one-way ANOVA. Correlation analysis was performed using Spearman correlation. P value < 0.05 was considered statistically significant.

Results

CPSF4 participated in ovarian cancer and promoted spheroid formation

As early as 2013, our previous work identified CPSF4 as an oncogenic driver in lung cancer, bringing this protein to the forefront of cancer research. (Chen, Guo et al. 2013) Over the ensuing decade, the aberrant upregulation of CPSF4 across a broad spectrum of human malignancies has been firmly established. (Zhang, Lin et al. 2021, Ge, Niu et al. 2025) We first examined CPSF4 protein expression in ovarian cancer cell lines by Western blotting. Compared with the normal ovarian surface epithelial cell lines HOSE and IOSE80, CPSF4 protein levels were markedly elevated in ovarian cancer cell lines (Fig. 1A). Notably, high CPSF4 expression tended to correlate with poorer clinical outcomes in patients with ovarian cancer. Overall survival (OS), recurrence-free survival (RFS), and post-progression survival (PPS)

are among the most widely employed endpoints, reflecting the full disease course, the post-treatment period, and the post-recurrence phase, respectively. Kaplan–Meier analyses demonstrated that elevated CPSF4 levels were significantly correlated with reduced overall survival (OS), recurrence-free survival (RFS) and shorter post-progression survival (PPS), suggesting that CPSF4 may contribute to disease aggressiveness and poor therapeutic response following tumor relapse (Fig. 1B).

Under stem cell–selective conditions, tumor cells were cultured as non-adherent spheroids, a model that faithfully recapitulated the advanced-stage characteristics of ovarian carcinoma cells in malignant ascites. Utilizing this approach, we successfully and efficiently isolated cancer stem cells (CSCs) from ovarian cancer cell lines.(Shi, Artemenko et al. 2025) Cancer stem cells (CSCs) from the SKOV-3 and HEYA8 cell lines demonstrated robust spheroid-forming ability and expressed higher levels of the stemness-associated markers β -catenin, Bmi-1, Nanog, and Oct4 than their non-cancer stem cells counterparts (NCSCs). Under normal differentiating conditions with the restoration of 10% fetal bovine serum (FBS), the spheroid cells re-adhered and resumed adherent growth, and the expression of the stem cell markers was markedly reduced in the differentiated cells, further demonstrating the plasticity of the undifferentiated phenotype of tumour cells (Fig. 1C). Considering the critical role of RNA processing regulators in maintaining cancer stemness and tumor aggressiveness, we investigated whether CPSF4 contributes to ovarian cancer progression and CSC-associated phenotypes. We showed a significant increase of CPSF4 in CSCs, whereas CPSF4 expressed little in NCSCs (Fig. 1D). To further characterize the cellular distribution of CPSF4 in ovarian cancer, single-cell RNA sequencing (scRNA-seq) data were analyzed (Fig. 1E). UMAP visualization revealed that CPSF4 expression was not uniformly distributed across cell populations but was predominantly enriched in malignant epithelial cell clusters. Notably, elevated CPSF4 expression was also observed in proliferative cell populations, suggesting a potential association with active cell-cycle status and stem-like features. In contrast, CPSF4 expression was relatively low in immune and stromal compartments, including lymphoid and myeloid cells. These findings indicated that CPSF4 upregulation is largely tumor cell–intrinsic and may be linked to stemness-associated phenotypes in ovarian cancer.

Inhibition of CPSF4 reduces olaparib resistance of cancer stem cells

To further elucidate the importance of CPSF4 in CSCs, we employed a short hairpin RNA (shRNA) specifically targeting CPSF4. Quantitative real-time PCR (qRT–PCR) analysis demonstrated that this shRNA efficiently knocked down CPSF4 expression (Fig. 2A). Our results showed that, compared with the nonspecific shRNA, CPSF4 shRNA not only led to a marked reduction in proliferation in NCSCs, but also significantly suppressed tumour spheroid formation in CSCs, which was consistent with our expectations (Fig. 2B). Unlike lymphatic and hematogenous metastasis, tumour–mesothelial interaction represents a key dissemination feature of ovarian cancer.(Bajwa, Kordylewicz et al. 2023, Mei, Chen et al. 2023) Mesothelial adhesion assays were employed to model tumour–mesothelial interactions. Using the human mesothelial cell line MeT5A, we demonstrated that CPSF4 silencing markedly impaired tumour cell adhesion to the mesothelial monolayer compared with control cells, thereby leading to a

substantially reduced intraperitoneal metastatic potential (Fig. 2C). In clinical practice, olaparib has been established as a standard-of-care therapy for ovarian cancer in both frontline and recurrent settings, significantly prolonging progression-free survival in patients with homologous recombination deficiency (HRD). However, the emergence of acquired or primary resistance to PARPi, such as olaparib, posed a significant challenge in the clinical management of ovarian cancer. (Wakefield, Nesic et al. 2019, Zou, Zhang et al. 2025) To investigate whether CPSF4 contributed to PARPi resistance, we examined the combined effect of CPSF4 knockdown and olaparib in CSCs and found that CSCs proliferation was markedly inhibited by either shRNA-mediated CPSF4 knockdown or olaparib alone; notably, their combined administration synergistically enhanced drug sensitivity, surpassing the effect observed with either treatment alone (Fig. 2D).

CPSF4 promoted the activation of the Ca(2+) signaling pathway

RNA sequencing predicts alterations in tumor cell signaling pathways by analyzing differential gene expression, pathway enrichment, and related approaches based on whole-transcriptome sequencing. As an indispensable tool, it closely links transcriptional alterations to functional signaling networks in cancer. (Liu, Yan et al. 2025, Shen, Li et al. 2025) To further delineate the impact of CPSF4 depletion on downstream gene regulation, we conducted RNA sequencing (RNA-seq)-based transcriptomic profiling of CPSF4-silenced ovarian cancer cells in two independent cell lines. A total of 682 genes were differentially expressed in SKOV3 cells, while 2,944 genes were altered in HEYA8 cells. Intersection analysis identified 104 genes that were consistently dysregulated in both cell lines, highlighting a core CPSF4-associated transcriptional signature (Fig. 3A). Based on the criteria of $|\log_2(\text{fold change})| > 1$, consistent direction of change, and statistical significance, CACNA2D2 was identified as the only gene meeting all requirements among the 104 candidate genes (Fig. 3B). Hierarchical clustering analysis of the DEGs uncovered a set of genes associated with the modulation of calcium channels, notably CACNA2D2, GRIN2C, and S100A5 (Fig. 3C). (Schafer, Fritschy et al. 2000, Chung, Marson et al. 2016) Collectively, the enrichment landscape underscored calcium signaling as a central node of transcriptional reprogramming, leading us to prioritize investigation of the calcium ion channel gene CACNA2D2 (Fig. 3D). Correlation analysis revealed a significant positive association between CPSF4 and CACNA2D2 expression ($R = 0.28$), indicating a moderate yet biologically relevant correlation. This relationship was highly statistically significant ($P = 4 \times 10^{-9}$), effectively excluding random variation as a contributing factor. Collectively, these findings suggest that elevated CPSF4 expression is accompanied by increased CACNA2D2 expression, supporting a potential regulatory or functional linkage between CPSF4 and CACNA2D2 in this context (Fig. 3E). Importantly, the positive association between CPSF4 and CACNA2D2 expression was not confined to ovarian cancer samples but was also reproducibly detected across normal human ovary tissues in the GTEx dataset, underscoring the robustness and biological relevance of this correlation (Fig. 3F). Finally, this finding was further validated by evidence that targeting CPSF4 led to a marked downregulation of CACNA2D2 mRNA expression (Fig. 3G).

CPSF4 and CACNA2D2 expressions showed a positive correlation in OvCa specimens

Integrative microarray-based gene expression analysis coupled with patient clinical and survival data is essential for identifying oncogenic signaling pathways with true biological and prognostic relevance clinically, enabling direct links between gene dysregulation, clinical characteristics and patient outcomes.(Berchuck, Iversen et al. 2009, Gyorffy, Lanczky et al. 2012) To investigate the clinical relevance of CPSF4 and CACNA2D2 in ovarian cancer, immunohistochemical (IHC) staining was performed in tumor tissues and corresponding normal samples. Quantitative analysis revealed that CPSF4 expression was significantly elevated in ovarian cancer tissues compared with normal controls (**P = 0.0373**; Fig. 4A). Representative IHC images further confirmed increased CPSF4 expression in tumor tissues relative to normal ovarian epithelium (Fig. 4B). To further assess the relationship between CPSF4 and CACNA2D2, their expression patterns were compared in ovarian cancer tissues. Samples with high CPSF4 expression consistently showed elevated CACNA2D2 levels, whereas tissues with low CPSF4 expression displayed correspondingly reduced CACNA2D2 staining (Fig. 4C). Taken together, these results indicated a positive correlation between CPSF4 and CACNA2D2 expression in clinical ovarian cancer specimens.

CPSF4 promoted tumor growth *in vivo* via regulation of CACNA2D2

To further validate the oncogenic role of CPSF4 *in vivo*, a subcutaneous xenograft mouse model was established using SKOV3 cells with CPSF4 knockdown or corresponding control cells. As shown in Fig. 5A, tumors derived from CPSF4-knockdown cells were markedly smaller than those in the control group. Consistently, longitudinal measurement of tumor volume demonstrated that CPSF4 depletion significantly suppressed tumor growth over time compared with the control group (Fig. 5B). At the experimental endpoint, tumor weights and sizes in the CPSF4-knockdown group were significantly reduced, confirming the inhibitory effect of CPSF4 loss on tumor progression. Quantitative analysis further verified a significant decrease in CPSF4 expression in xenograft tumors derived from CPSF4-knockdown cells (Fig. 5C). To explore the downstream mechanism *in vivo*, immunohistochemical (IHC) staining was performed. As shown in Fig. 5D, CPSF4 depletion led to a marked reduction in CACNA2D2 expression in tumor tissues, consistent with the *in vitro* findings. Collectively, these results demonstrate that CPSF4 promotes ovarian cancer tumor growth *in vivo*, at least in part through the upregulation of CACNA2D2.

Discussion

Ovarian cancer is characterized by a unique transcoelomic metastatic pattern, and accumulating evidence suggests that anchorage-independent survival and spheroid formation are key determinants of disease dissemination and therapeutic failure. In this study, we demonstrated that CPSF4 is significantly upregulated in stemness-enriched ovarian cancer spheroids and functions as a critical regulator of cancer stem-like properties.

Emerging studies have increasingly implicated RNA-processing factors in tumor progression; however, their contribution to cancer stemness remains poorly understood. CPSF4, as a core component of the pre-mRNA cleavage and polyadenylation machinery, has been previously linked to tumor proliferation and metastasis in several malignancies. Our findings extend these observations by demonstrating that CPSF4 is preferentially enriched in ovarian cancer stem-like cells and is required for maintaining their self-renewal capacity, spheroid formation, and resistance to olaparib. These results suggest that CPSF4 may function as a key post-transcriptional regulator that supports stemness-associated transcriptional programs under non-adherent conditions.

Mechanistically, our study identified that CACNA2D2 is a critical downstream effector of CPSF4. RNA-seq revealed that knockdown of CPSF4 simultaneously downregulated CACNA2D2 in independent ovarian cancer cell lines. CACNA2D2 encodes an auxiliary subunit of voltage-gated calcium channels, playing a crucial role in modulating calcium influx and intracellular Ca^{2+} homeostasis. Calcium signaling pathways are widely recognized as core regulators of cancer cell survival, metabolic adaptation, and stemness maintenance. Intriguingly, the role of CACNA2D2 in cancer appears to be context dependent. Our results supported that CACNA2D2 is an oncogene in ovarian cancer, and its upregulation is associated with increased stemness and poor clinical outcomes. This context-specific function may reflect differences in tumor microenvironment, cellular state (e.g., stem-like vs differentiated), or calcium signaling dynamics, highlighting the complexity of ion channel regulation in cancer biology. Our findings suggested that CPSF4 promotes the expression of CACNA2D2, thereby enhancing calcium influx and sustaining signaling pathways that are beneficial for stem cell-like phenotypes and therapeutic resistance.

We further confirmed that targeting CPSF4 significantly enhances the sensitivity of ovarian cancer stem-like cells to the PARP inhibitor olaparib, which is currently widely used in clinical treatment. Given that PARP inhibitor resistance remains a major challenge in the clinical treatment of ovarian cancer, our results suggest that the CPSF4–CACNA2D2 axis may represent a previously unknown PARP inhibitor resistance mechanism. Simultaneously, CPSF4 inhibition combined with olaparib treatment showed a synergistic effect in reducing tumor spheroid formation and cell viability, suggesting that targeting this pathway may have potential therapeutic value. From a clinical perspective, our data further demonstrated a significant positive correlation between CPSF4 and CACNA2D2 expression in ovarian cancer tissues, and the expression of both genes was associated with poor prognosis.

Despite these findings, several limitations need to be noted. First, our study primarily relies on two ovarian cancer cell lines, which may not fully capture the heterogeneity. Validation in more models, including organoids, is needed. Second, although we demonstrate a regulatory relationship between CPSF4 and CACNA2D2, the precise molecular mechanism remains to be elucidated. Third, rescue experiments are needed to further confirm the functional dependency of this axis.

In summary, as shown in the schematic model (Fig. 6), our study reveals a novel CPSF4–CACNA2D2–calcium signaling pathway axis that promotes cancer stemness maintenance and olaparib resistance in

ovarian cancer.

Conclusion

In conclusion, this study demonstrates that CPSF4 promotes stemness maintenance and olaparib resistance in ovarian cancer through the CACNA2D2-mediated calcium signaling pathway under non-adherent conditions. Our findings reveal, for the first time, a critical role of CPSF4 in sustaining stemness-associated olaparib resistance, highlighting its potential as a therapeutic target for overcoming ovarian cancer metastasis and drug resistance.

Declarations

Authors' contributions

Canhui Yi: carried out all the experiments, writing – original, data curation, formal analysis. Yuan Zhu: writing – original, data curation, formal analysis. Bo Wang: study designing, writing – review and editing. All authors have read and approved the final version of the manuscript.

Funding

Sanming project of Medicine in Shenzhen (No. SZSM202411015) and Research Start-up Fund of Post-doctoral of SAHSYSU (No. ZSQYRSFPD0058)

Data availability

All source data for this work are available upon reasonable request.

Ethics approval

All animal experiments were conducted using principles approved by the Guidelines for the Care and Use of Laboratory Animals of Guangzhou Medical University. The protocol was approved by the Ethics Committee of Guangzhou Medical University.

Competing interests

The authors declare no competing interests.

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Figures

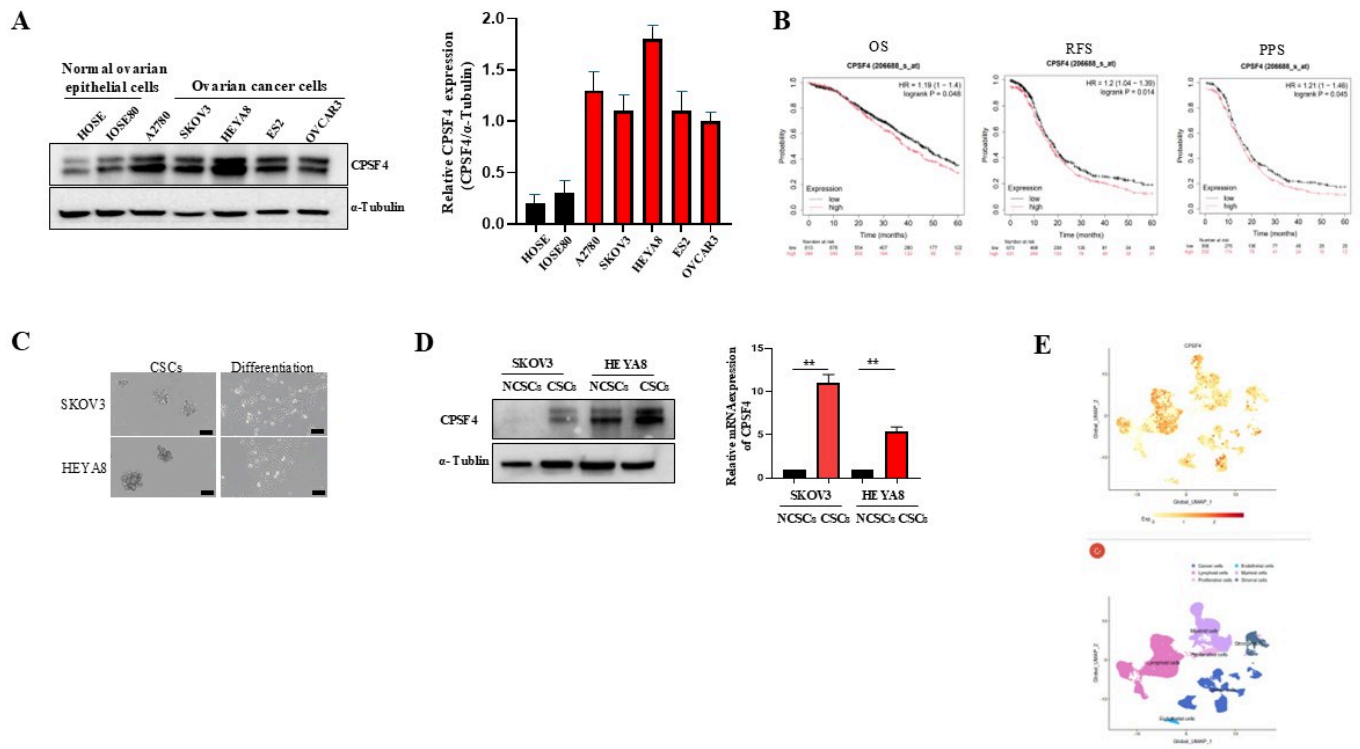


Figure 1

CPSF4 is upregulated in ovarian cancer and enriched in ovarian cancer stem-like cells.

(A) The expression of CPSF4 was determined by Western blot analysis in normal ovarian epithelial cells and ovarian cancer cell lines. α -Tubulin was used as the loading control. Quantification of CPSF4 expression normalized to α -Tubulin is shown on the right. **(B)** Kaplan–Meier survival analysis of ovarian cancer patients stratified by CPSF4 expression levels, including overall survival (OS), relapse-free survival (RFS), and post-progression survival (PPS). **(C)** Representative morphological images showing ovarian cancer stem-like cells (CSCs) cultured under non-adherent spheroid-forming conditions and their re-differentiation after being transferred back to normal adherent culture medium. **(D)** Western blot analysis of CPSF4 expression in non-cancer stem cells (NCSCs) and CSCs derived from SKOV3 and HEYA8 ovarian cancer cell lines. **(E)** CPSF4 profiles across different cell populations was determined by single-cell sequencing (sc-seq) analysis. Data are presented as mean $\pm$ SD from three independent experiments. * $P < 0.05$, ** $P < 0.01$.

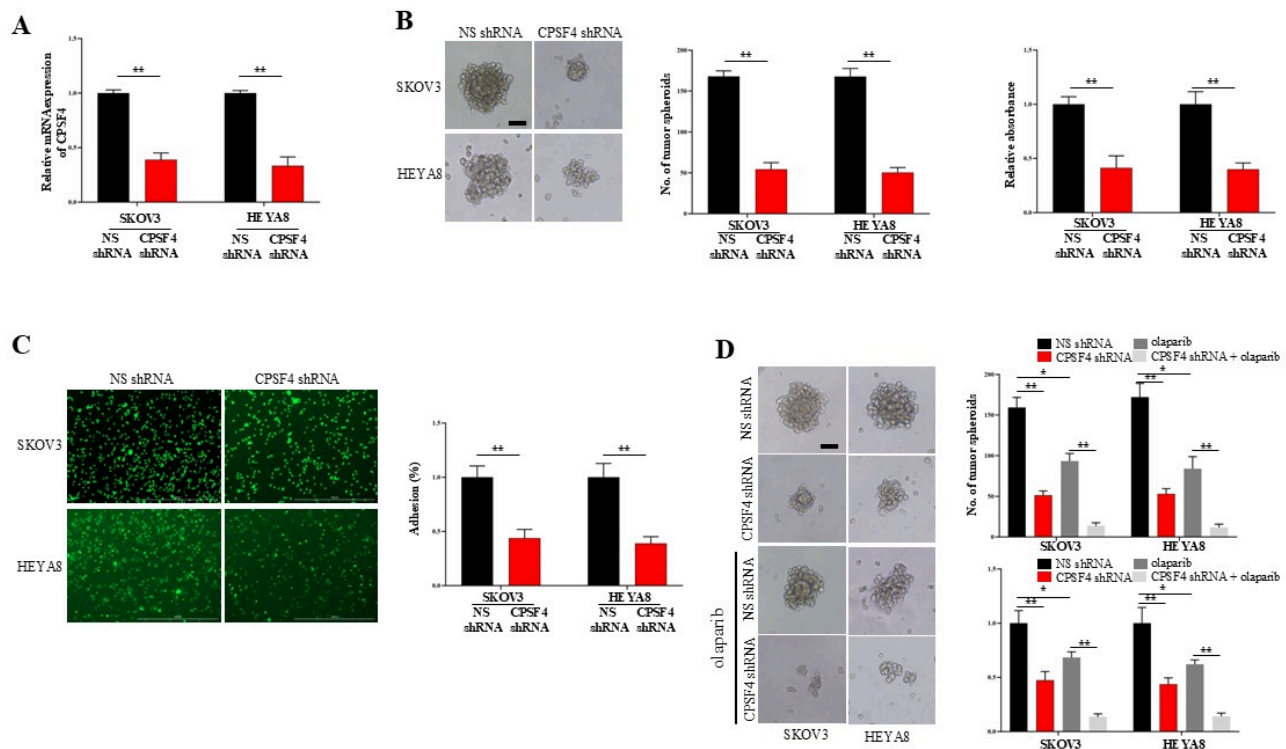


Figure 2

CPSF4 knockdown suppressed stemness, adhesion capacity, and resistance to olaparib of ovarian cancer cells.

(A) CPSF4 knockdown efficiency was confirmed by quantitative RT-PCR analysis in SKOV3 and HEYA8 cells transfected with CPSF4 shRNA compared with negative control (NS shRNA). **(B)** The number and cell viability of tumor spheroids formed was confirmed by photographed and MTT assay in SKOV3 and HEYA8 cells. **(C)** The depletion of CPSF4 significantly decreases the adhesive capacity of ovarian cancer cells to mesothelium by cell adhesion assays. **(D)** Cells were transfected with CPSF4 shRNA alone or in combination with olaparib for 48 h. The number of spheroids generated was photographed and counted. Scale bar, 50 μ m. Cell viability was determined by MTT assay. * $P < 0.05$, ** $P < 0.01$.

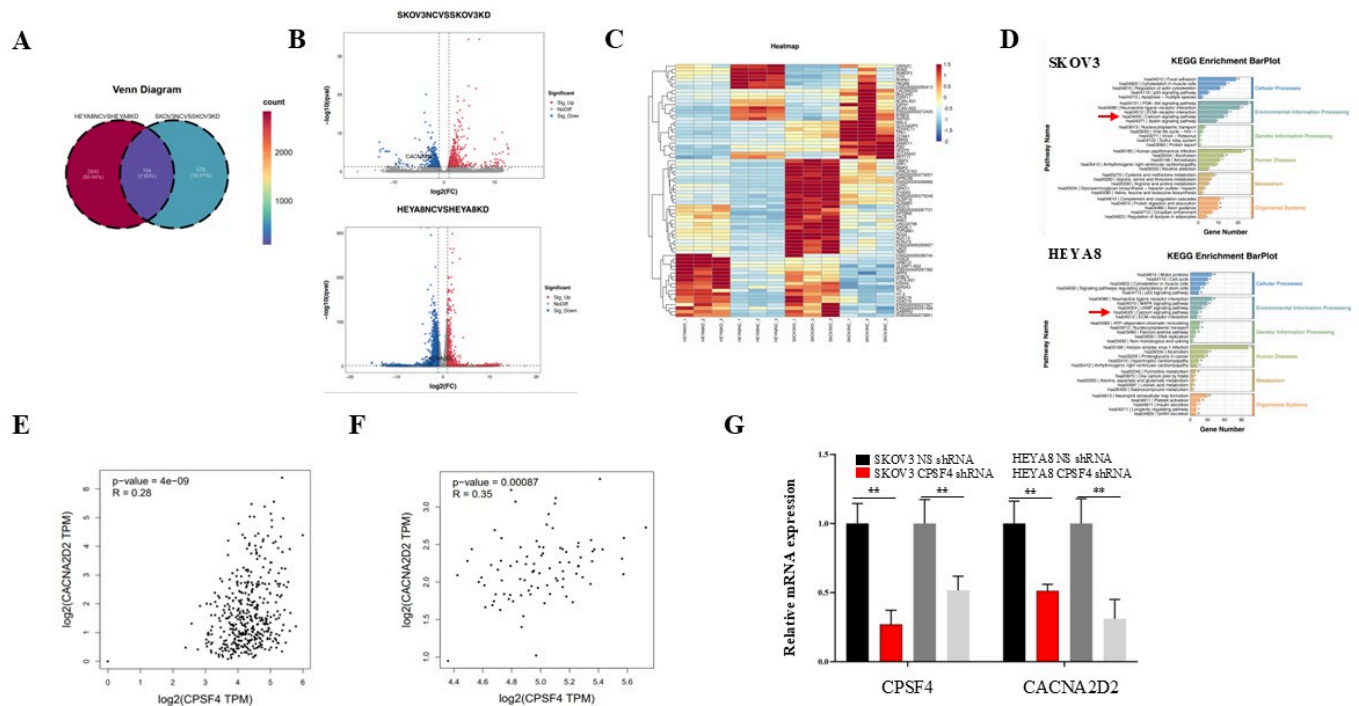


Figure 3

RNA-seq and characterization of differential gene expression in SKOV3 and HEYA8 knockdown vs negative control.

(A) The commonly regulated targets of CPSF4 presented by intersected genes across different ovarian cancer cell lines in Venn diagram. **(B)** Each differentially expressed genes (DEGs) was showed by Volcano plot. Upregulated genes was represented by red and downregulated genes by blue dots. **(C)** Heatmap displaying Hierarchical clustering of significantly altered genes was displayed by Heatmap following CPSF4 depletion. **(D)** Signaling pathways affected by CPSF4 knockdown were indicated by KEGG pathway enrichment analysis. **(E)** CPSF4 and CACNA2D2 expression was analyzed by Spearman correlation in ovarian cancer tissues. **(F)** CPSF4 and CACNA2D2 expression was analyzed by Spearman correlation in normal ovarian tissues. Correlation coefficients (r) and P values are indicated in the plots. **(G)** Quantitative RT-PCR validation of selected candidate genes identified from RNA-seq analysis. Data are presented as mean $\pm$ SD from three independent experiments. * $P < 0.05$, ** $P < 0.01$.

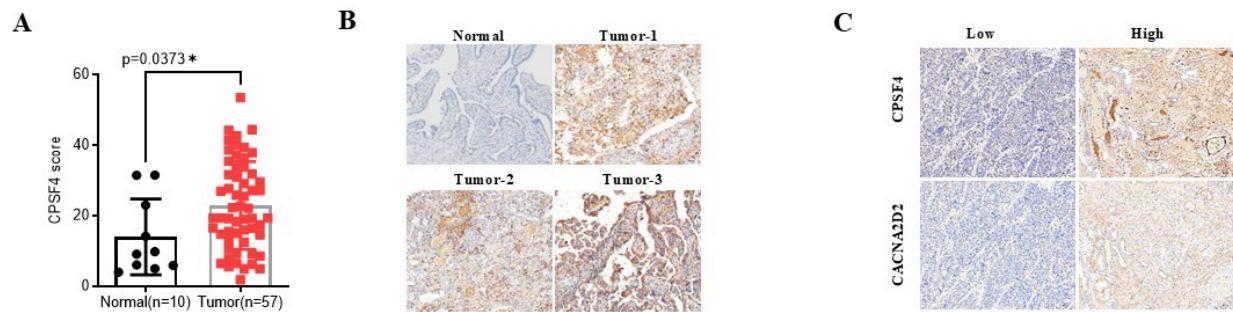


Figure 4

CPSF4 was highly expressed in ovarian cancer tissues and positively correlated with CACNA2D2 expression.

(A)Immunohistochemical (IHC) scoring of CPSF4 expression in normal ovarian tissues (n = 10) and ovarian cancer tissues (n = 57). **(B)** CPSF4 expression was shown by representative IHC images in normal ovarian tissue and ovarian cancer tissues with different expression levels (low and high). **(C)** Correlation analysis of CPSF4 and CACNA2D2 expression in ovarian cancer tissues.

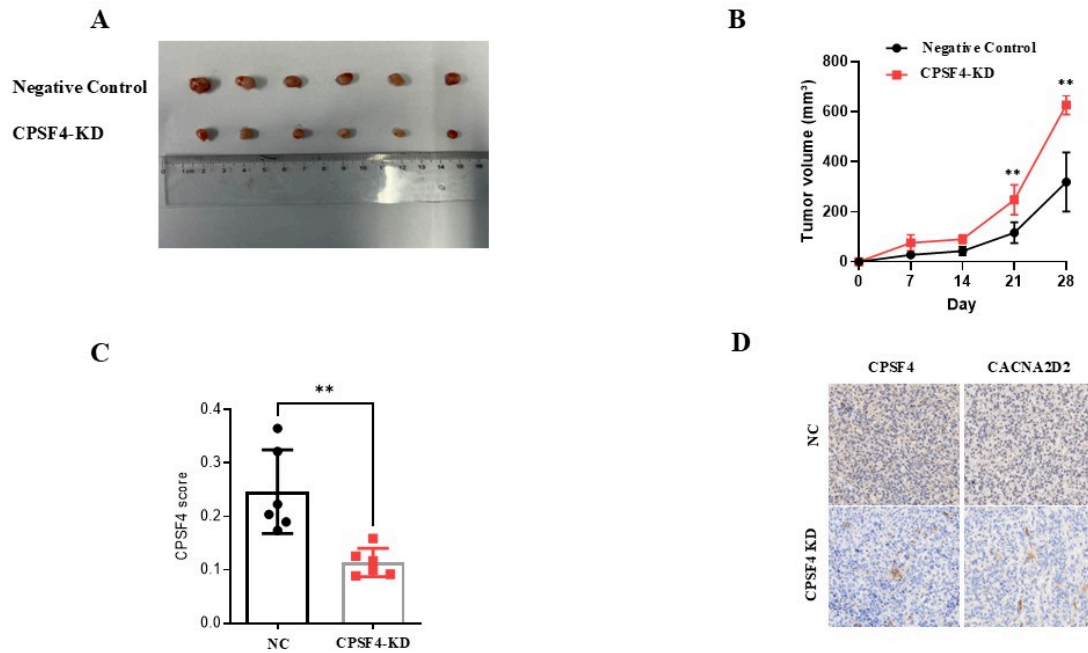


Figure 5

Tumor growth was promoted by CPSF4 expression *in vivo*.

(A) Representative images of tumors isolated from mice in the NC and CPSF4-KD groups. **(B)** Tumor growth curves of xenograft tumors formed by control (NC) and CPSF4-knockdown ovarian cancer cells. Tumor volumes were measured at the indicated time points. **(C)** Quantification of CPSF4 expression scores in xenograft tumors derived from NC and CPSF4-KD cells. **(D)** IHC analysis of CPSF4 and CACNA2D2 protein levels in xenograft tumor tissues. Data are presented as mean $\pm$ SD. ** $P < 0.01$.

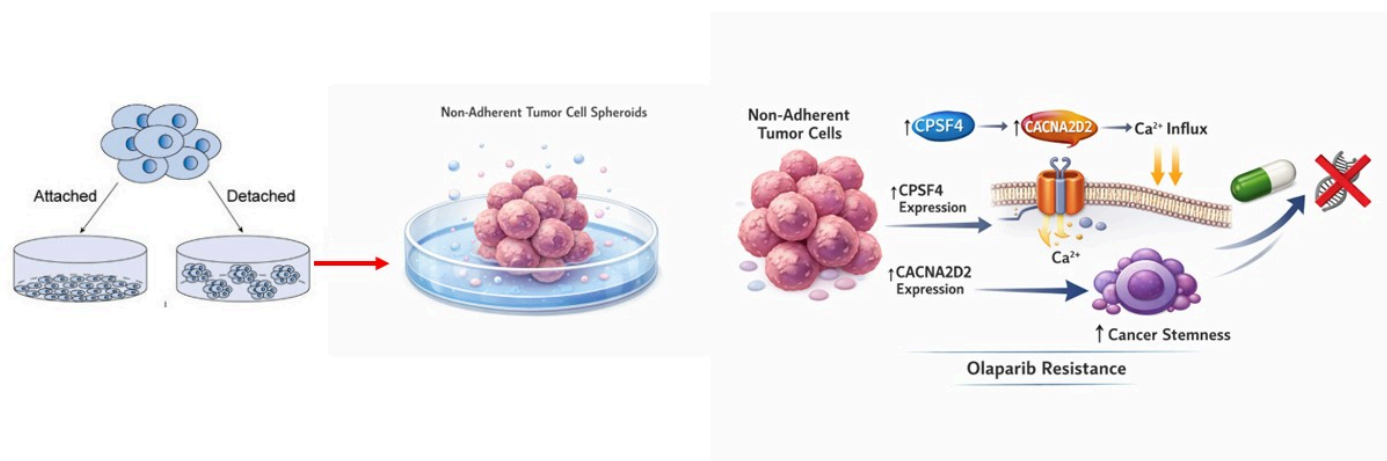


Figure 6

Schematic model illustrating the role of CPSF4 in promoting ovarian cancer stemness and olaparib resistance.

Under non-adherent conditions, CPSF4 expression was upregulated in ovarian cancer cells, which in turn induced CACNA2D2 expression and enhanced calcium influx. The activation of calcium signaling promoted cancer stem-like properties and contributed to olaparib resistance.

Supplementary Files

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