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Interference with CHD1L inhibits the malignant progression and enhances cisplatin sensitivity of ovarian cancer cells by binding PLK1

Kun Qiao[1,4*†], Yuanxiazi Guan[2†] and Wenjing Xing[3]

Abstract

Background Chromodomain helicase/ATPase DNA-binding protein 1-like gene (CHDIL) is an oncogene with abnormal expression in ovarian cancer (OC), but its regulatory role in the malignant biological properties of OC cells and its mechanisms have not been reported.

Methods In this study, CHD1L and polo-like Kinase 1 (PLK1) expression in OC tissues and OC cell lines was analyzed. After CHD1L silencing, CAOV-3 cell proliferation and apoptosis were detected by CCK8 assay, EDU and TUNEL staining. Flow cytometry was used to detect cell cycle. CCK8 assay and TUNEL were used to detect the role of CHD1L in the sensitivity of OC cells to cisplatin. In addition, the abilities of CAOV-3 cell migration and invasion were evaluated using wound healing assay and transwell assay. Next, the binding between CHD1L and PLK1 was investigated using co-immunoprecipitation assay. Then, PLK1 was overexpressed to perform the rescue experiments to analyze the regulation mechanism of CHD1L on OC development and cisplatin sensitivity. Moreover, the transplantation tumor model of CAOV-3 cells in nude mice was established to explore the antineoplastic effect of CHD1L downregulation in vivo.

Results CHD1L was highly expressed in OC tissues and OC cells. Interference with CHD1L significantly inhibited proliferation, promoted apoptosis, induced cycle arrest, suppressed migration and invasion as well as enhanced the sensitivity of CAOV-3 cells to cisplatin. Additionally, CHD1L could interact with PLK1. PLK1 upregulation restored the impacts of CHD1L knockdown on the proliferation, apoptosis, cycle arrest, migration, invasion and the sensitivity of OC cells to cisplatin. It could be also found that CHD1L knocked down limited the tumor volume, downregulated PLK1, Ki67 and cleaved caspse3 expression.

Conclusion Taken together, interference with CHD1L inhibited the malignant progression and enhanced cisplatin sensitivity of OC cells by binding PLK1.

Keywords Ovarian cancer, CHD1L, PLK1, Proliferation, Apoptosis, Cell cycle arrest, Cisplatin sensitivity

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Introduction

The incidence rate of ovarian cancer (OC) ranks the third among female reproductive system malignant tumors, and the mortality rate ranks the first among gynecological malignant tumors. In 2020, there were 313,959 new cases of OC in the world, and 55,342 new cases in China, accounting for 17.62%. OC led to 207,252 deaths, accounting for 4.7% of female cancer-related deaths [1, 2]. Due to its high mortality rate, OC is commonly considered as one of the most malignant tumors in women. Screening for early diagnosis of OC is of great significance for the detection and control of the disease [3]. In addition, because of the insidious onset of OC, most patients with OC are diagnosed in advanced stages, and advanced tumors are prone to become resistant to chemotherapy drugs such as cisplatin [4]. Therefore, overcoming chemotherapy resistance has become a key factor to improve the outcomes of OC patients. Understanding the molecular mechanism of cisplatin resistance in OC has extremely important value for exploring effective targeted therapy and developing new drugs to reverse drug resistance.

Chromodomain helicase/ATPase DNA-binding protein 1-like gene (CHDIL) is a newly discovered oncogene located in 1q21 through high-throughput sequencing, which is positively associated with the progression of many solid tumors [5, 6]. Studies have found that CHD1L also plays an important role in transcriptional regulation, maintenance of chromosome integrity and DNA repair; therefore, the positive expression of CHD1L is closely related to the proliferation, invasion, metastasis and prognosis of malignant tumors [7, 8]. It has been shown that the positive expression of CHD1L is closely related to the process of OC metastasis and CHD1L may be used as a new biomarker for the prognosis of OC patients [9]. In addition, the expression of CHD1L is increased in human OC tissues, and it can promote the invasion and migration of OC cells [10]. However, the effect of CHD1L on the proliferation, apoptosis and cell cycle of OC cells and whether it can enhance the sensitivity of chemotherapy remain unknown.

Through high-throughput sequencing, the BioGrid database, hitpredict database and HDOCK show that CHD1L can combine with polo-like Kinase 1 (PLK1). A previous study has shown that PLK1 inhibition can suppress the invasion and migration of glioma cells and promote cell apoptosis [11]. Inhibition of PLK1 enhances the sensitivity of cisplatin to oral squamous cell carcinoma [12]. In addition, the expression of PLK1 is increased in OC tissues, and higher expression of PLK1 predicts the poorer prognosis of OC patients [13]. Therefore, this study aimed to explore whether CHD1L could affect the proliferation, apoptosis, cycle arrest, migration and invasion of OC cells by combining with PLK1.

Materials and methods

Bioinformatics analysis

CHD1L and PLK1 expression in human OC tissues ($n = 374$) and normal ovarian tissues ($n = 133$) was analyzed using TNMPLOT database (https://tnmplot.com/). The combination between CHD1L and PLK1 was predicted by BioGrid database (https://thebiogrid.org/), hitpredict database (http://www.hitpredict.org/) and HDOCK (http://hdock.phys.hust.edu.cn/).

Cell culture

Human normal ovarian epithelial cells IOSE80 cells (cat.no BNCC358126), OC cell lines OVCAR3 (cat. no BNCC339586), CAOV-3 (cat.no BNCC101010) and SCOV3 (cat.no BNCC310551) cells were obtained from BeNa Culture Collection (Henan, China). All cells were cultured in DMEM medium (Gibco) containing 10% fetal bovine serum (FBS, Gibco), 100 IU/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich). Cells were grown in sterile culture dishes at 37 °C with 5% CO_2. To detect the sensitivity of OC cells to cisplatin, CAOV-3 cells were treated with different concentrations of cisplatin (0, 20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml, HY-17394, MedChemExpress) for 48 h.

Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was extracted from sample cells with TRIzol reagent (Invitrogen, Carlsbad, CA). 1 μg of total RNA was reverse transcribed into first-strand complementary DNA (cDNA) with a commercial RevertAid™ cDNA Synthesis kit (Bio-Rad). qPCR was performed on an ABI PRISM 7900HT (Applied Biosystems; Life Technologies; Thermo Fisher Scientific, Inc.) using SYBR Premix Ex Taq™ (Takara Bio, Inc.) or Taqman probes (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The relative expression levels of mRNA were normalized to GAPDH expression, and fold changes in expression were calculated using the $2^{-\Delta\Delta Ct}$ method. The sequences of all primers used for RT-qPCR are as follows: CHD1L: forward: 5'-GGGAAGACCTGC CAGATTTGCT-3', reverse: 5'-TTTCACTCGCCTCAG CAGAAA-3'; PLK1: forward: 5'- TGACTCAACACGCC TCATCC-3', reverse: 5'- CTCGTCGATGTAGGTCACG G-3'; GAPDH: forward: 5'-AATGGGCAGCCGTTAGG AAA-3', reverse: 5'- GCGCCCAATACGACCAAATC-3'.

Western blot

Cells were digested in RIPA buffer (Shanghai Absin Biotechnology Co., Ltd.) on ice. A bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.) was used to detect the protein concentrations. Subsequently, 30 μg protein was separated by 10% SDS-PAGE gel and transferred to PVDF membranes (Sigma-Aldrich). Then the membranes were blocked with 5% bovine serum albumin

(BSA) for 2 h and were incubated at 4 °C overnight with primary antibodies. After that, the membranes were incubated with goat anti-rabbit horseradish-peroxidase-conjugated secondary antibody (1:5000, Abcam) for 2 h at room temperature. Finally, the protein blots were visualized using an enhanced chemiluminescence detection system (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. GAPDH was used as the internal control.

Small-interfering RNA transfection

CHD1L small interfering RNAs (siRNA-CHD1L-1 and siRNA-CHD1L-2) and negative control siRNA (siRNA-NC), PLK1 overexpression plasmid (Ov-PLK1) and the empty vector plasmid (Ov-NC) were provided by Shanghai GenePharma Co., Ltd. (Shanghai, China). Above recombinants were transfected into CAOV-3 cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) strictly in accordance with the recommended specifications. The transfection efficacy was determined using RT-qPCR after 48 h.

Cell counting Kit-8 (CCK8) assay

CAOV-3 cells (2×10^3 cells/well) were seeded into 96-well plates, and 10 μl CCK8 solution (Beyotime Technology, Shanghai, China) was added into each well to incubate cells for additional 2 h at 37 °C. The optical density was measured at 450 nm using a microplate reader.

EDU staining assay

EDU staining was used to analyze CAOV-3 cell proliferation according to the standard protocol. Briefly, CAOV-3 cells were transfected and then incubated with EDU (20 mmol/L) for 2 h. The cells were fixed with 4% paraformaldehyde for 20 min at room temperature and permeated with 0.5% Triton X-100 for 10 min at room temperature. Subsequently, CAOV-3 cells were incubated with Click reaction solution in the dark for 30 min and DAPI dye was applied for the counterstaining of cell nucleus. The images were obtained using a fluorescence microscope (Olympus Corporation).

TUNEL assay

CAOV-3 cells (2×10^4 cells/well) were seeded into 6-well plates and subjected to indicated treatment. Subsequently, the cells were fixed with 4% paraformaldehyde at 37˚C for 15 min and then incubated with TUNEL solution for 1 h at 37˚C. After that, cells were stained with 3,3-diaminobenzidine (Sigma-Aldrich) for 10 min at room temperature according to the manufacturer's protocol. Cell nuclei were stained with 0.1 μg/ml DAPI for 5 min and nuclear DNA fragmentation was assessed using the DeadEnd™ Fluorometric TUNEL system (Promega Corporation). Finally, cells in five randomly selected fields were observed under an Olympus IX71 fluorescence microscope (Olympus Corporation).

Flow cytometry

The treated CAOV-3 cells at 70–80% confluence were digested into a single-cell suspension, fixed in 70% ethanol, stained with propidium iodide (PI), and analyzed by flow cytometry. In addition, the percentages of cells within each phase of the cell cycle were analyzed with ModFit version 4.0 (Verity Software House, Inc., Topsham, ME, USA) and CellQuest version 5.1 (Thermo Fisher Scientific, Inc.).

Wound healing assay

CAOV-3 cells (5×10^5) were seeded onto 6-well plates and grown to 90% confluence. Subsequently, cells were scratched by a 200-μl pipette tip. After that, the medium was replaced by serum-free medium and the PBS-rinsed cells were nourished for 24 h. The wounds were captured at 0 and 24 h using an inverted light microscope (Olympus Corporation). Cell migration rate was evaluated using ImageJ software.

Transwell assay

Following the re-suspension in serum-starved DMEM, CAOV-3 cells were added into the upper chambers with Matrigel pre-treatment while DMEM containing 10% FBS was placed on the lower chambers. After nourishing for 24 h, cells passing the membranes were exposed to fixing solution and labeled with 0.1% crystal violet solution. Images were captured using a light microscope (Olympus Corporation) and cell counts were calculated using ImageJ software.

Co-immunoprecipitation (co-IP)

CAOV-3 cells were lysed and the supernatant was removed. Following, the proteins were incubated with 5 μg anti-CHD1L or anti-PLK1 and a total of 10 μl protein A/G agarose beads (cat. no. sc-2003; Santa Cruz Biotechnology, Inc.) overnight at 4℃. Isotype-matched IgG was used as negative control. After that, beads were decoupled by SDS loading buffer boiling. In addition, the input we take is half of the total lysate. The samples were analyzed by western blot.

Analysis of tumorigenicity in nude mice

BALB/c nude mice (5 weeks old) purchased from Sipple-Bikai Laboratory Animal Co., Ltd (Shanghai, China) were applied for the evaluation of the tumor growth of CAOV-3 cells in vivo. They were bred under SPF conditions, which maintained a controlled environment (a light/dark cycle for 12 h, at 22–24 °C, and relative humidity of 60%). Lentivirus expressing small hairpin RNA (shRNA) targeting CHD1L (Lv-shRNA-CHD1L)

and its negative control (Lv-shRNA-NC) were produced and concentrated with a lentivirus packaging system (Clontech, Palo Alto, CA, USA). Animals were arbitrary assigned into three groups: control, Lv-shRNA-NC and Lv-shRNA-CHD1L ($n = 5$ per group). BALB/c nude mice were injected with about 200 μL (1×10^6) CAOV-3 cells transfected with Lv-shRNA-NC or Lv-shRNA-CHD1L under the skin of the right flank. The tumor growth was observed every two days. On the 21th day, all mice were sacrificed, and tumor tissues were taken out and photographed. The animal experiments were approved by the Animal Ethics Committee of Shandong Provincial Maternal and Child Health Care Hospital.

Immunohistochemical analysis

The tumor-bearing tissues were fixed with 4% paraformaldehyde and then dehydrated and embedded in paraffin. After that, 5-μm-thick sections were sequentially immersed in xylene, anhydrous ethanol and alcohol to dewax. 3% hydrogen peroxide and 5% BSA were used to block the endogenous peroxidase activity and nonspecific antigens respectively. The sections were incubated with Ki67 or cleaved caspase3 antibodies at 4 °C overnight. After incubating with secondary antibody, the slides were developed DAB and counterstained with hematoxylin. Images were captured under a light microscope (Olympus, Tokyo, Japan).

Statistical analysis

All statistical analyses were performed using GraphPad 8.0 software (GraphPad Software Inc., USA). The experimental data are represented as mean ± SD. Discrepancies among multiple groups were checked by one-way ANOVA analysis with a post-hoc Tukey's test. Statistical significance was set at $P < 0.05$.

Results

The expression of CHD1L is increased in OC tissues and cell lines

TNMPLOT database was used to analyzed CHD1L expression in OC tissues and normal ovarian tissues. As shown in Fig. 1A, CHD1L expression was notably upregulated in OC tissues compared with that in the normal ovarian tissues. RT-qPCR and Western blot were used to detect the expression of CHD1L in OC cell lines, and the results showed that the expression of CHD1L in OC cell lines was significantly increased compared with IOSE80 cells (Fig. 1B and C). Among them, CHD1L had the highest expression in CAOV-3 cells, so CAOV-3 cells were selected for the following experiments. The above results showed that the expression of CHD1L was increased in OC tissues and cell lines.

Interference with CHD1L inhibits proliferation, promotes apoptosis and induces cycle arrest in OC cells

Next, siRNA-CHD1L was transfected into CAOV-3 cells, and the transfection efficiency was detected by RT-qPCR and Western blot. siRNA-CHD1L-1 was selected for subsequent experiments for its more prominent interference efficacy (Fig. 2A and B). CAOV-3 cells were divided into control, siRNA-NC and siRNA-CHD1L groups. CCK8 assay results showed that cell proliferation in the siRNA-CHD1L group was significantly decreased by 40% compared with that in the siRNA-NC group (Fig. 2C). EDU staining showed that the inhibition of CHD1L significantly decreased cell proliferation (Fig. 2D). TUNEL and Western blot results showed that compared with siRNA-NC group, cell apoptosis was significantly increased by 25% in siRNA-CHD1L group (Fig. 2E), accompanied by a decrease in BCL2 expression and an increase in Bax and cleaved caspase3 expression (Fig. 2F). Subsequently, flow cytometry was used to detect cell cycle, and the results showed that after inhibiting the expression of CHD1L, the number of cells in G0/G1 phase was decreased while that of G2/M phase was increased (Fig. 2G). The expression of cycle-related proteins CDK1 and CyclinB1 was detected by Western blot. The results showed that the expression of CDK1 and CyclinB1 in the siRNA-CHD1L group was significantly decreased compared with that in the siRNA-NC group (Fig. 2H). The results indicated that interference with CHD1L inhibited proliferation, promoted apoptosis and induced cycle arrest in OC cells.

Interference with CHD1L suppresses the migration and invasion of OC cells

The effects of CHD1L silencing on the migration and invasion of CAOV-3 cells were analyzed using wound healing- and transwell assays. As displayed in Fig. 3A and B, compared with the control group, CHD1L knockdown led to the significant decrease in the migration rate of CAOV-3 cells and the number of invasive CAOV-3 cells. Consistently, notably downregulated MMP2 and MMP9 expression levels were observed in the siRNA-CHD1L group (Fig. 3C). These data revealed that CHD1L deficiency suppressed the migration and invasion of OC cells.

Interference with CHD1L promotes the sensitivity of OC cells to cisplatin

OC cell lines OVCAR3, SCOV3 and CAOV-3 were treated with different concentrations of cisplatin. As shown in Fig. 4A, the viability of OVCAR3, SCOV3 and CAOV-3 cells decreased significantly with increasing cisplatin concentrations. Among the three cell lines, CAOV-3 cells showed the most significant decrease in cell activity. Subsequently, CAOV-3 cells transfected with siRNA-CHD1L were treated with different

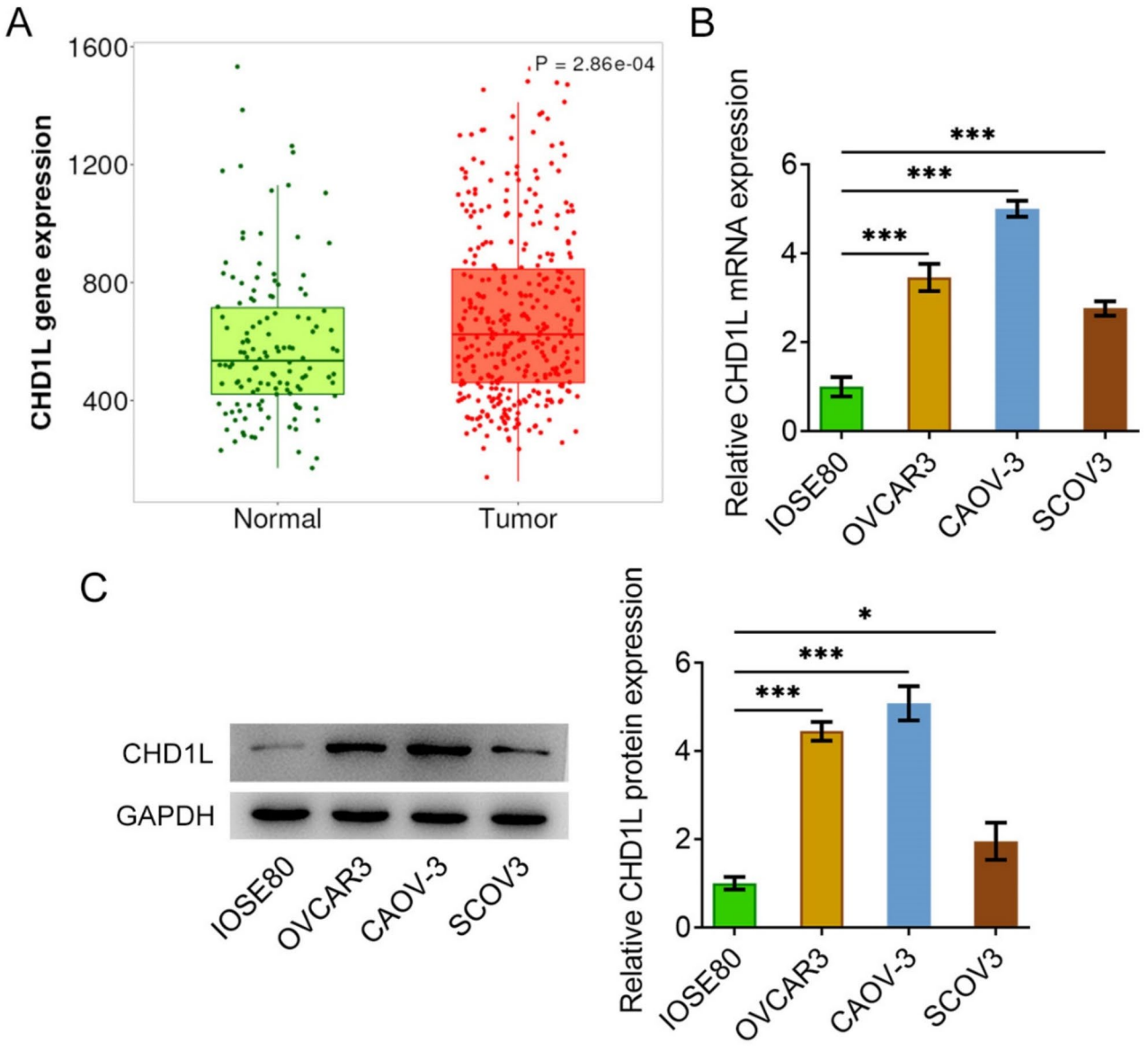

Fig. 1 The expression of CHD1L was increased in OC tissues and cell lines. **A**. CHD1L expression in human OC tissues ($n = 374$) and normal ovarian tissues ($n = 133$) was analyzed using TNMPLOT database. RT-qPCR (**B**) and western blot (**C**) detected the expression of CHD1L in OC cell lines OVCAR3, CAOV-3 and SCOV3. $^{*}P < 0.05$, $^{***}P < 0.001$

concentrations of cisplatin, and the cell activity was detected by CCK8 assay. The results showed that after the inhibition of CHD1, the cell viability was significantly decreased by cisplatin with a concentration of 20–40 μg/ml compared with siRNA-NC group (Fig. 4B). Cisplatin with a concentration of 20 μg/ml was selected for subsequent experiments. As shown in Fig. 4C and D, apoptosis was notably increased in Cisplatin + siRNA-CHD1L group relative to the Cisplatin + siRNA-NC group. To sum up, CHD1L knockdown promoted the sensitivity of CAOV-3 cells to cisplatin.

Interference with CHD1L inhibits PLK1 expression in OC cells

The BioGrid database, hitpredict database and HDOCK showed that CHD1L could combine with PLK1 (Fig. 5A-C). Results of TNMPLOT database analysis indicated that PLK1 expression was notably upregulated in OC tissues compared with that in the normal ovarian tissues (Fig. 5D). Next, the expression of PLK1 in OC cell lines was examined, and the results showed that PLK1 expression was abnormally elevated in OC cell lines, especially in CAOV-3 cells (Fig. 5E and F). Subsequently, it was found that the inhibition of CHD1L in CAOV-3 cells significantly inhibited PLK1 expression (Fig. 5G). Co-IP

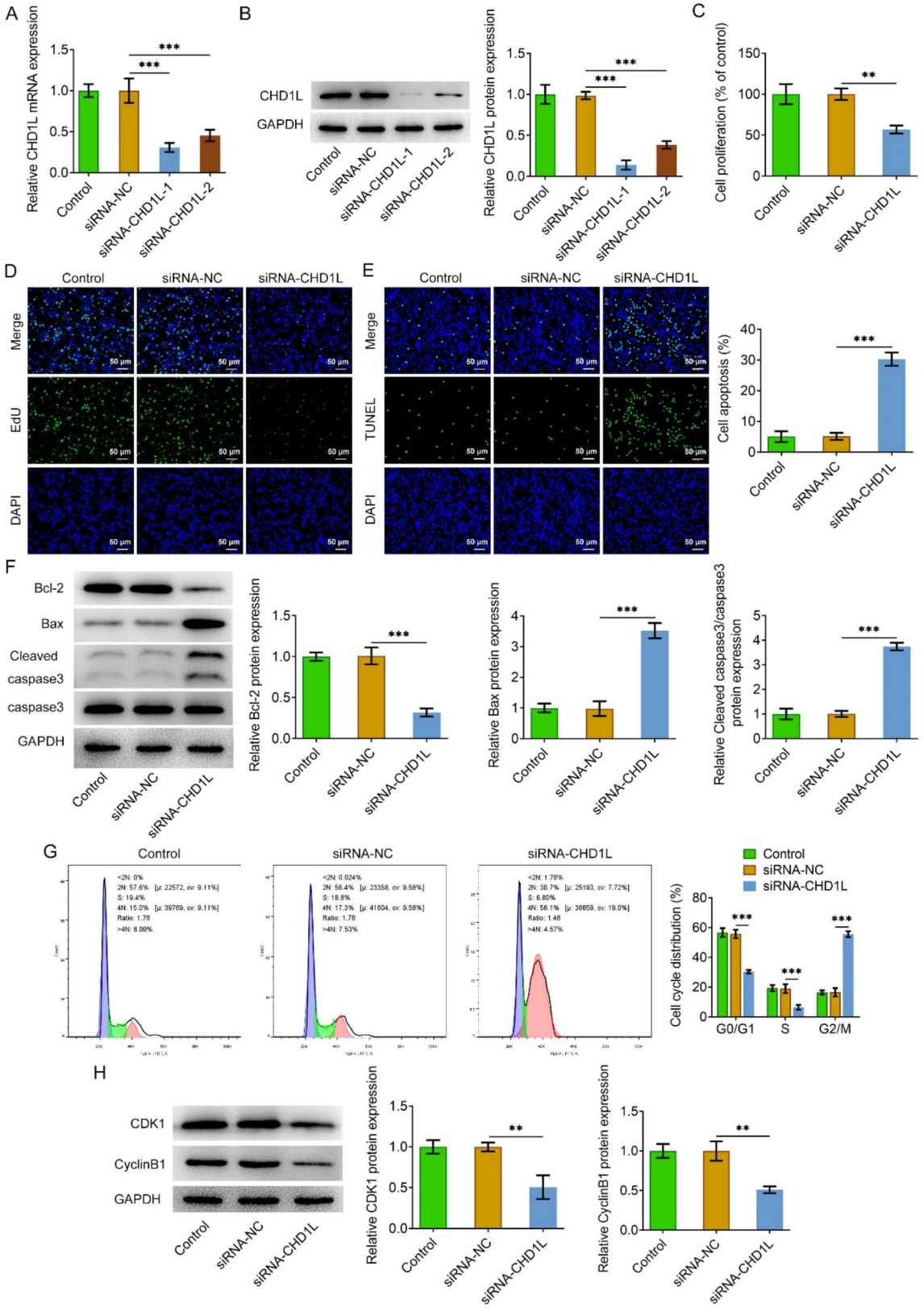

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Fig. 2 Interference with CHD1L inhibited the proliferation, promoted the apoptosis and induced cycle arrest in OC cells. siRNA-CHD1L was transfected into CAOV-3 cells and the transfection efficiency was detected using RT-qPCR (**A**) and western blot (**B**). **C**. CCK8 assay detected the proliferation of CAOV-3 cells transfected with siRNA-CHD1L. **D**. EDU staining detected the proliferation of CAOV-3 cells transfected with siRNA-CHD1L. **E**. TUNEL assay detected the apoptosis of CAOV-3 cells transfected with siRNA-CHD1L. **F**. Western blot detected the expression of apoptosis-related proteins Bcl-2, Bax, Cleaved caspase3 and caspase3 in CAOV-3 cells transfected with siRNA-CHD1L. **G**. Cycle distribution of CAOV-3 cells transfected with siRNA-CHD1L was determined by flow cytometry. **H**. Western blot detected the expression of cycle-related proteins CDK1 and CyclinB1 in CAOV-3 cells transfected with siRNA-CHD1L. $^{**}P < 0.01$, $^{***}P < 0.001$

experiments showed that CHD1L could bind to PLK1 (Fig. 5H). Above results revealed that interference with CHD1L inhibited PLK1 expression in OC cells.

Interference with CHD1L inhibits the proliferation, promotes apoptosis, induces cycle arrest and suppresses migration and invasion of OC cells by binding PLK1 s

The PLK1 overexpression plasmid was constructed and the transfection efficiency of Ov-PLK1 was detected by RT-qPCR and Western blot (Fig. 6A and B). CAOV-3 cells were divided into control, siRNA-CHD1L, siRNA-CHD1L + Ov-NC, and siRNA-CHD1L + Ov-PLK1 groups. CCK8 assay and EDU staining results showed that compared with the siRNA-CHD1L + Ov-NC group, the cell proliferation of the siRNA-CHD1L + Ov-PLK1 group was significantly increased (Fig. 6C and D). TUNEL and Western blot results showed that overexpression of PLK1 could significantly reverse the promotive effect of CHD1L depletion on cell apoptosis (Fig. 6E and F). Flow cytometry and Western blot results showed that overexpression of PLK1 could significantly reverse the cell cycle arrest induced by CHD1L inhibition (Fig. 6G and H). Besides, as comparison to the siRNA-CHD1L + Ov-NC group, the further PLK1 overexpression promoted the migration and invasion of CAOV-3 cells, accompanied with upregulated MMP2 and MMP9 expression (Fig. 7A-C). These data showed that interference with CHD1L inhibited the proliferation, promoted the apoptosis, facilitated cycle arrest and suppressed migration and invasion of OC cells by binding PLK1.

Interference with CHD1L promotes the cisplatin sensitivity of OC cells by binding PLK1

CCK8 assay was used to detect the viability of CAOV-3 cells treated with different concentrations of cisplatin. The results showed that the cell viability in siRNA-CHD1L + Ov-PLK1 group was significantly increased compared with that of siRNA-CHD1L + Ov-NC group upon the treatment with 20–40 μg/ml cisplatin (Fig. 8A). Cisplatin at a concentration of 20 μg/ml was selected for subsequent experiments. The cells were divided into siRNA-CHD1L, Cisplatin, Cisplatin + siRNA-CHD1L, Cisplatin + siRNA-CHD1L + Ov-NC and Cisplatin + siRNA-CHD1L + Ov-PLK1 groups. TUNEL results showed that Cisplatin treatment notably elevated the apoptosis of CAOV-3 cells compared with siRNA-CHD1L group (Fig. 8B). Additionally, relative to the Cisplatin group, the further CHD1L deficiency also resulted in the increased apoptosis of CAOV-3 cells. Moreover, cell apoptosis in Cisplatin + siRNA-CHD1L + Ov-PLK1 group was significantly decreased compared with the Cisplatin + siRNA-CHD1L + Ov-NC group. The data showed that interference with CHD1L promoted the cisplatin sensitivity of OC cells through PLK1.

CHD1L knockdown suppresses the development of subcutaneous CAOV-3 tumors in nude mice by downregulating PLK1 expression

The transplantation tumor model of CAOV-3 cells in nude mice was established to analyze the antineoplastic effect of CHD1L knockdown in vivo. As exhibited in Fig. 9A and B, CHD1L silencing notably decreased the tumor volume relative to the negative control group. Remarkably downregulated CHD1L and PLK1 levels were observed in the Lv-shRNA-CHD1L group (Fig. 9C). Besides, results of immunohistochemical analysis suggested that CHD1L knockdown significantly decreased Ki67 expression and increased cleaved caspase3 expression (Fig. 9D). These results revealed that CHD1L knockdown suppressed the development of subcutaneous CAOV-3 tumors by downregulating PLK1.

Discussion

As is known to all, the pathogenesis of OC is complex. In addition, drug resistance of OC is a difficult treatment problem affecting the prognosis of patients. The pathogenesis and mechanism of OC drug resistance is a multi-factorial process. A large number of studies have confirmed that changes in oncogenes and tumor suppressor genes affect tumor progression [14–16]. At the same time, advances in molecular genetics and biology have greatly accelerated the identification of molecular markers of cancer, which is beneficial to the diagnosis and treatment of OC [17, 18]. Therefore, it is very important to study the underlying molecular mechanism for overcoming drug resistance and inhibiting the occurrence and development of OC.

CHD1L is an oncogene that is amplified and expressed in various cancers such as colorectal cancer, gastric cancer, glioma, and liver cancer. Zhang et al. [3] have proved that CHD1L is overexpressed in hepatocellular carcinoma and the inhibition of CHD1L can significantly inhibit the migration and proliferation of hepatocellular

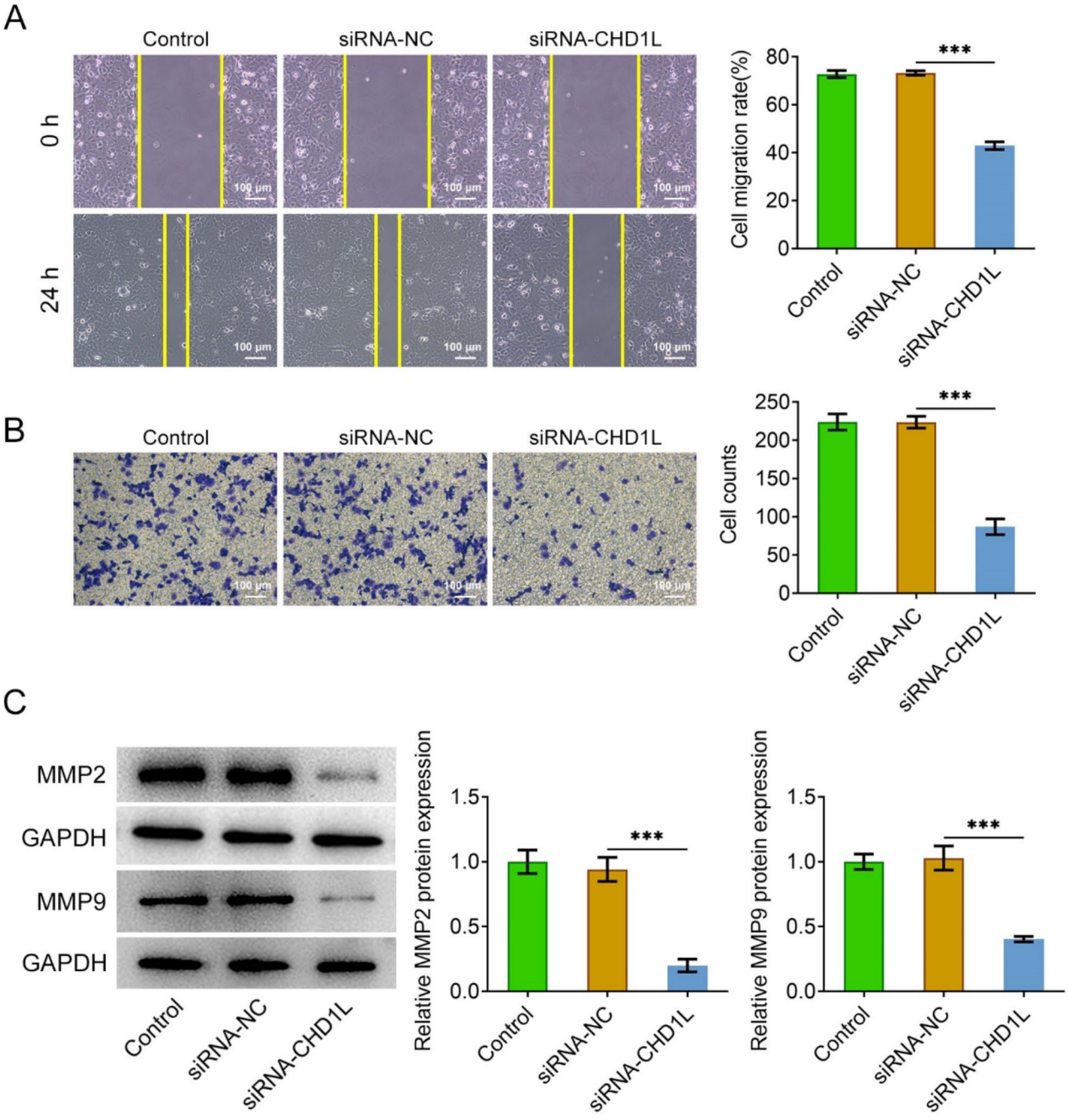

Fig. 3 Interference with CHD1L suppressed the migration and invasion of OC cells. (**A**) The migration of CAOV-3 cells transfected with siRNA-CHD1L was tested by wound healing assay. (**B**) The invasion of CAOV-3 cells transfected with siRNA-CHD1L was evaluated using transwell assay. (**C**) Western blot detected MMP2 and MMP9 expression in CAOV-3 cells transfected with siRNA-CHD1L. $^{***}P < 0.001$

carcinoma cells [19]. CHD1L is overexpressed in colorectal cancer tissues, and CHD1L overexpression can promote G1/S phase transition and inhibit apoptosis, and is correlated with tumor size, tumor invasion depth and tissue stage, proving that CHD1L plays an important role in the invasion and metastasis of colorectal cancer [7]. CHD1L silencing significantly inhibits the proliferation, invasion, and migration of BGC-823 gastric cancer cells and induces apoptosis. Knockdown of CHD1L may present a novel strategy for the treatment of gastric cancer [20]. In our experiments, we verified that the expression of CHD1L was abnormally increased in OC tissues and OC cell lines OVCAR3, CAOV-3 and SCOV3, especially in CAOV-3 cells. Therefore, we selected CAOV-3 cells for follow-up experiments. We found that inhibiting CHD1L could significantly inhibit proliferation, promote

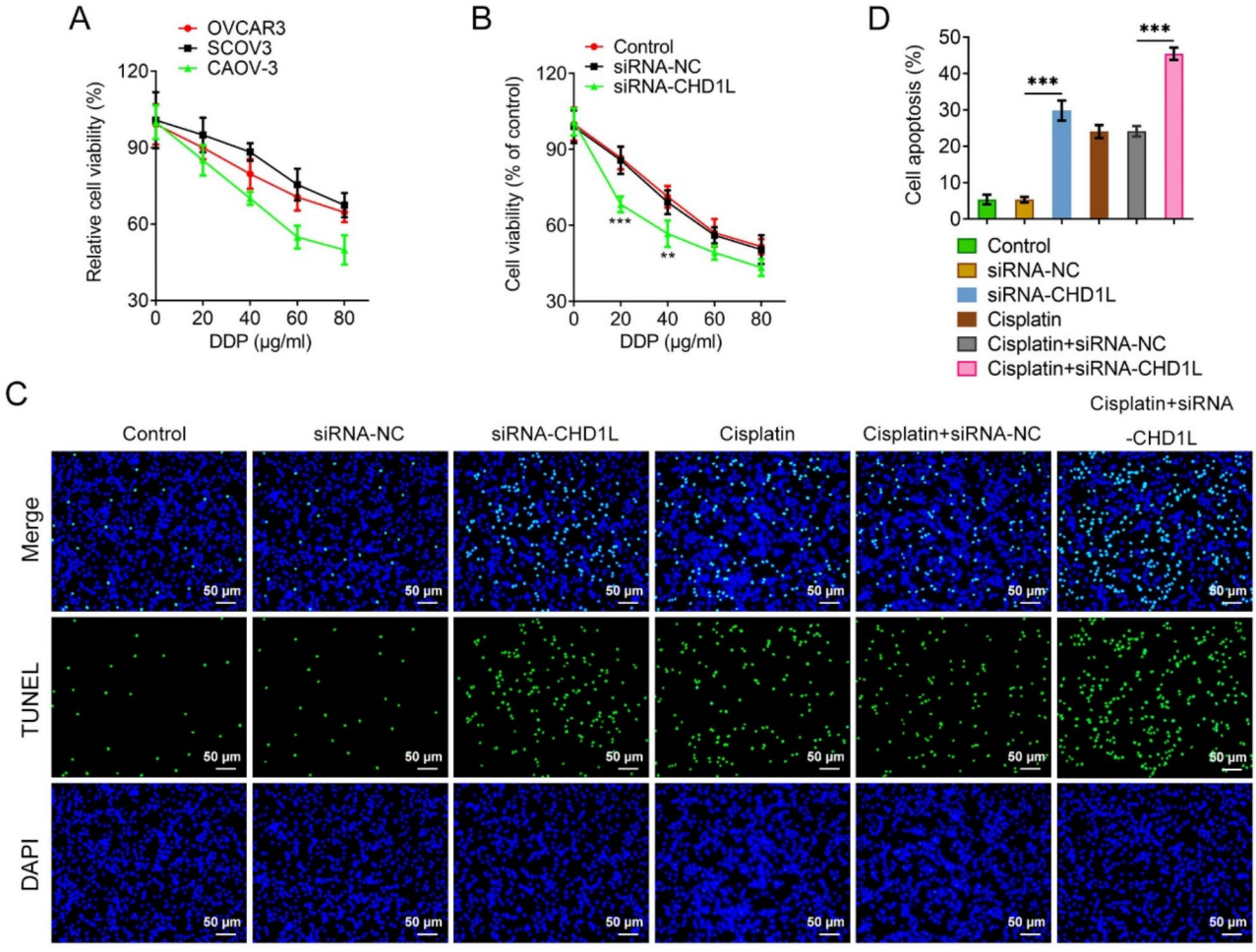

Fig. 4 Interference with CHD1L promoted the sensitivity of OC cells to cisplatin. (**A**) OC cell lines OVCAR3, SCOV3 and CAOV-3 were treated with different concentrations of cisplatin, and cell viability was assessed using CCK8 assay. (**B**) the transfected CAOV-3 cells were treated with varying concentrations of cisplatin, and then CCK8 detected the viability CAOV-3 cells. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. siRNA-NC. C and D. TUNEL assay detected the apoptosis of CHD1L-silenced CAOV-3 cells with cisplatin treatment. $^{***}P < 0.001$

apoptosis, induce cycle arrest and suppress migration and invasion of OC cells. The in vivo experiments also supported that CHD1L silencing suppressed the growth of subcutaneous CAOV-3 tumors in nude mice.

Platinum-based chemotherapy after tumor reduction is the first choice for patients with advanced OC and recurrent OC, but almost all patients with recurrent OC eventually develop platinum-based resistance. Therefore, it is important to explore the mechanism of cisplatin resistance. Previous study has shown that CHD1L promotes cisplatin resistance by upregulating ABCB1-NF-κB axis in human non-small cell lung cancer [21]. Inhibition of CHD1L can reduce the proliferation and cisplatin resistance of osteosarcoma [22]. However, the role of CHD1L in cisplatin resistance in OC has not been reported so far. Our study showed that interfering with CHD1L significantly enhanced the sensitivity of OC cells to cisplatin.

Next, we investigated the regulatory mechanism of CHD1L on OC proliferation, apoptosis and cycle, and the mechanism of its sensitivity to cisplatin. We found that interference with CHD1L expression in OC cells significantly inhibited PLK1 expression. The expression of PLK1 in OC cells was also abnormally elevated. In addition, we demonstrated that CHD1L could bind to PLK1 in OC cells. PLK1 can regulate multiple stages of cell mitosis and affect the progression of cell cycle, thereby playing a role in tumor formation. PLK1 is a promising clinical therapeutic target for a variety of malignancies, including OC [23]. The expression of PLK1 is significantly increased in early OC patients, and PLK1 expression is related to the prognosis and survival of OC patients [24]. The inhibition of PLK1 can downregulate the expression of proteins related to the development of OC, thus playing a significant role in OC treatment [25]. In our experiments, we found that the overexpression of PLK1 could reverse the impacts of CHD1L silencing on the proliferation, apoptosis, cycle arrest, migration and invasion of OC cells. In addition, cisplatin resistance in gastric

A

Interactor	Role	Organism	Experimental Evidence Code	Dataset	Throughput	HTP Score	Curated By
PLK1	BAIT	H. sapiens	Affinity Capture-MS	Hein MY (2015)	High	-	BioGRID

B

Interactor	Experiments	Homologs	Category	Interaction Score	Confidence
PLK1 Serine/threonine-protein kinase PLK1	1	2	High-throughput	0.536	High

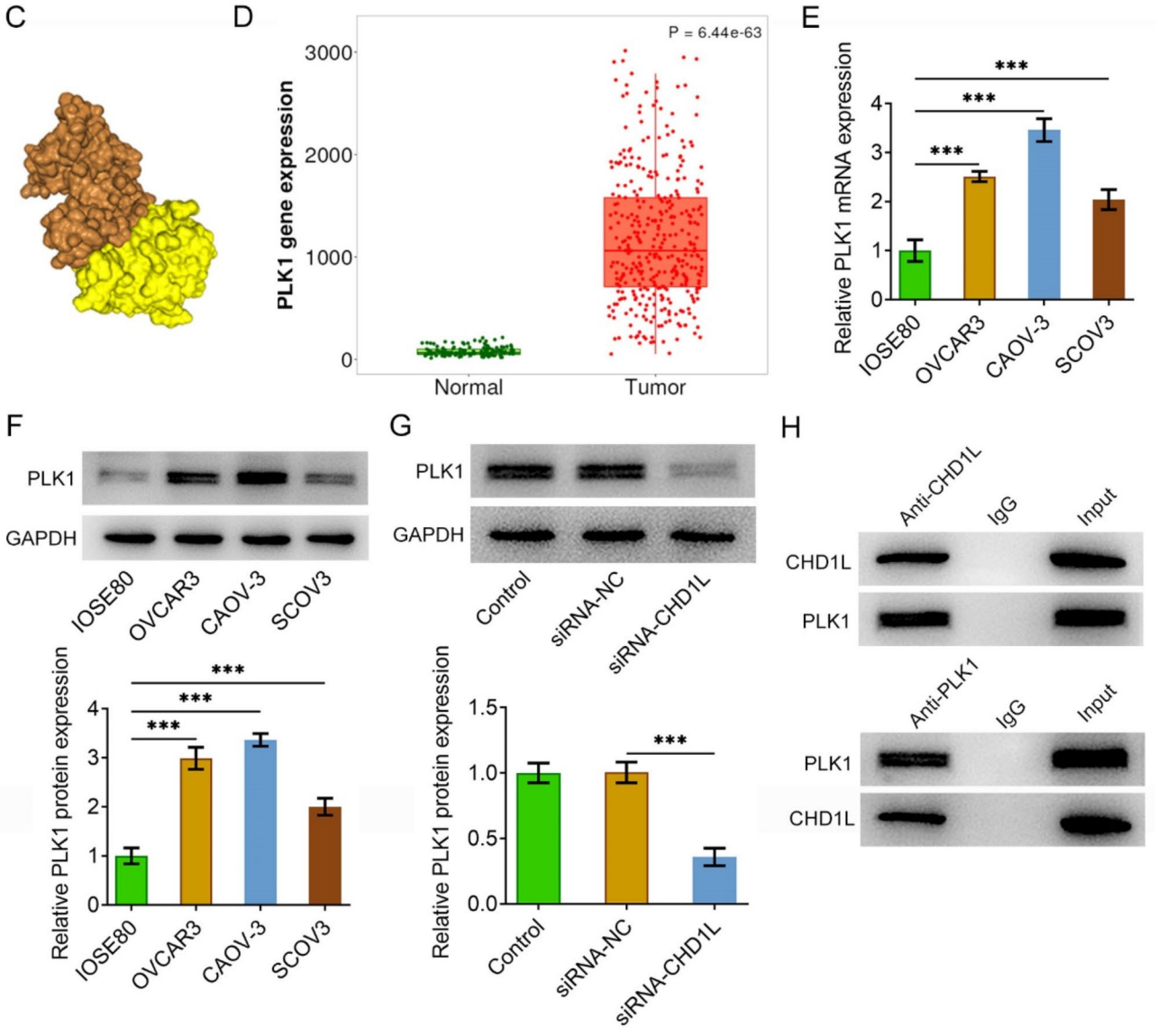

Fig. 5 Interference with CHD1L inhibited PLK1 expression in OC cells. The (**A**) BioGrid database, (**B**) hitpredict database and (**C**) HDOCK showed that CHD1L could combine with PLK1. **D**. PLK1 expression in human OC tissues ($n=374$) and normal ovarian tissues ($n=133$) was analyzed using TNMPLOT database. RT-qPCR (**E**) and Western blot (**F**) detected the expression of PLK1 in OC cell lines OVCAR3, CAOV-3 and SCOV3. **G**. Western blot detected the expression of PLK1 in CAOV-3 cells transfected with siRNA-CHD1L. **H**. Interaction between CHD1L and PLK1 was analyzed using Co-IP assay. $^{***}P<0.001$

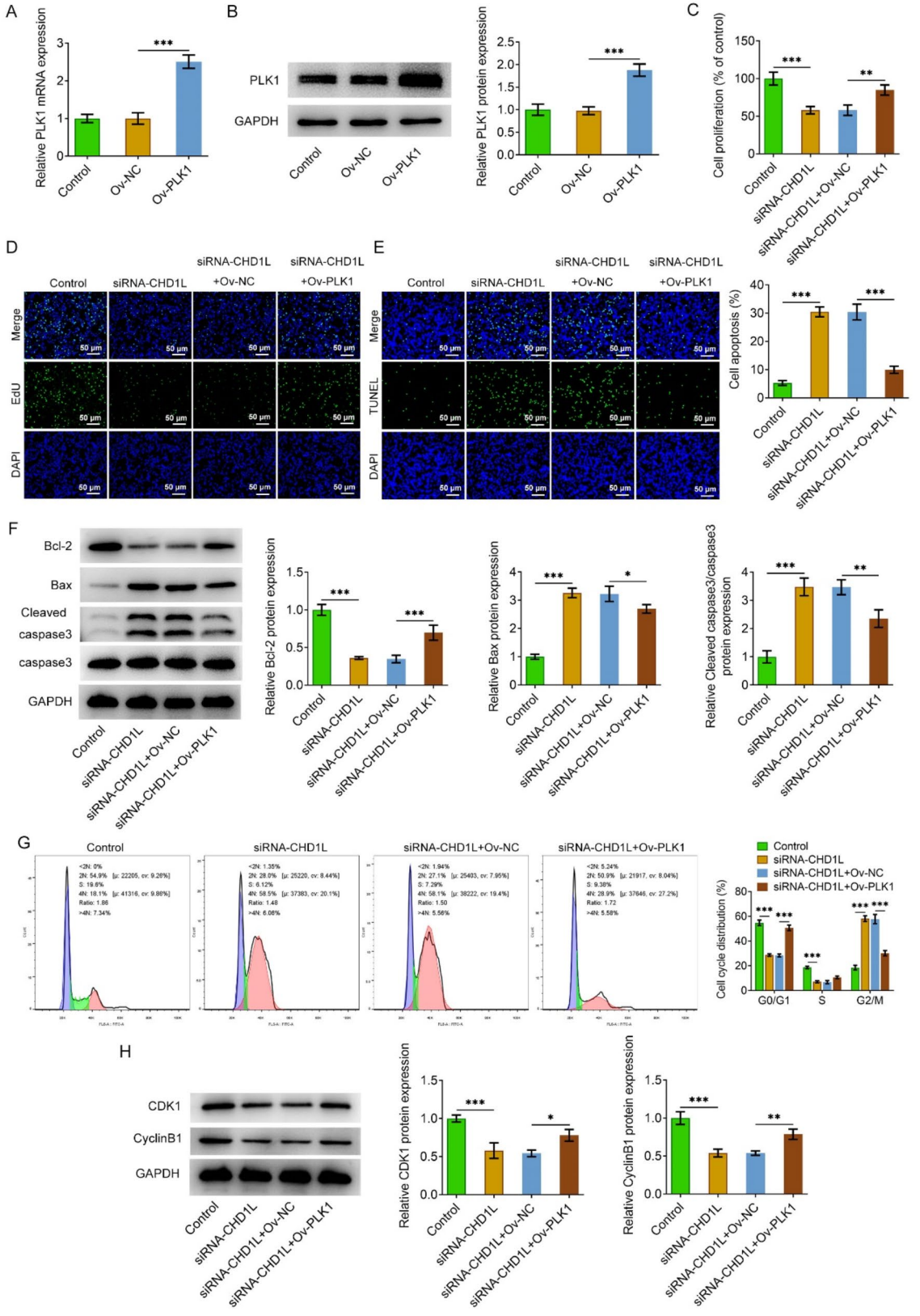

Fig. 6 (See legend on next page.)

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Fig. 6 Interference with CHD1L inhibited the proliferation, promoted the apoptosis and induced cycle arrest in OC cells by binding PLK1. The PLK1 overexpression plasmid was constructed and the transfection efficiency of Ov-PLK1 was detected by RT-qPCR (**A**) and Western blot (**B**). **C** and **D**. EDU staining detected the proliferation of CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L. **E**. TUNEL assay detected the apoptosis of CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L. **F**. Western blot detected the expression of apoptosis-related proteins Bcl-2, Bax, Cleaved caspase3 and caspase3 in CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L. **G**. Cycle distribution of CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L was determined by flow cytometry. **H**. Western blot detected the cycle-related proteins CDK1 and CyclinB1 in CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

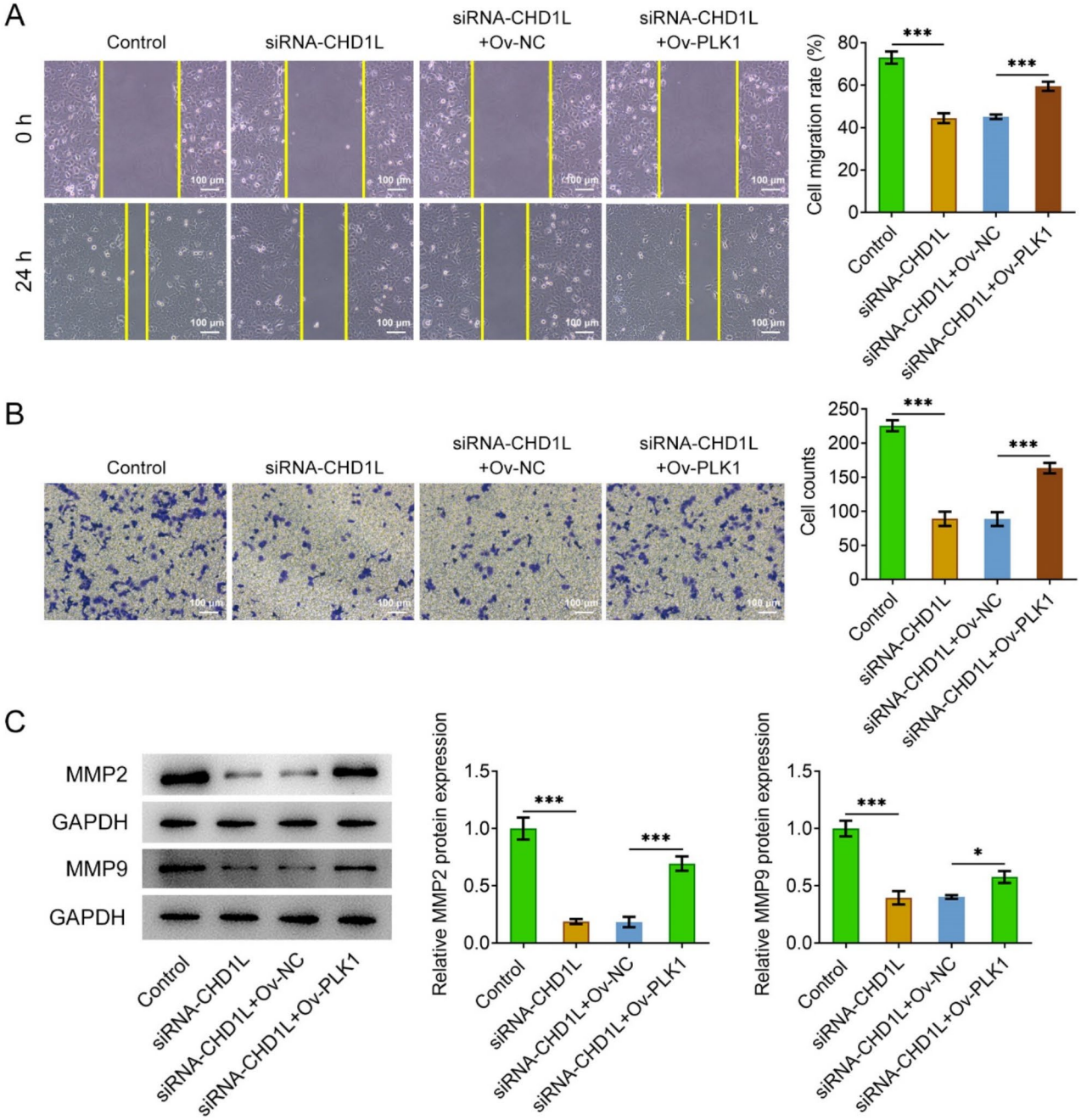

Fig. 7 Interference with CHD1L suppressed the migration and invasion of OC cells by binding PLK1. (**A**) The migration of CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L was tested by wound healing assay. (**B**) The invasion of CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L was evaluated using transwell assay. (**C**) Western blot detected MMP2 and MMP9 expression in CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L. $^{*}P<0.05$, $^{***}P<0.001$

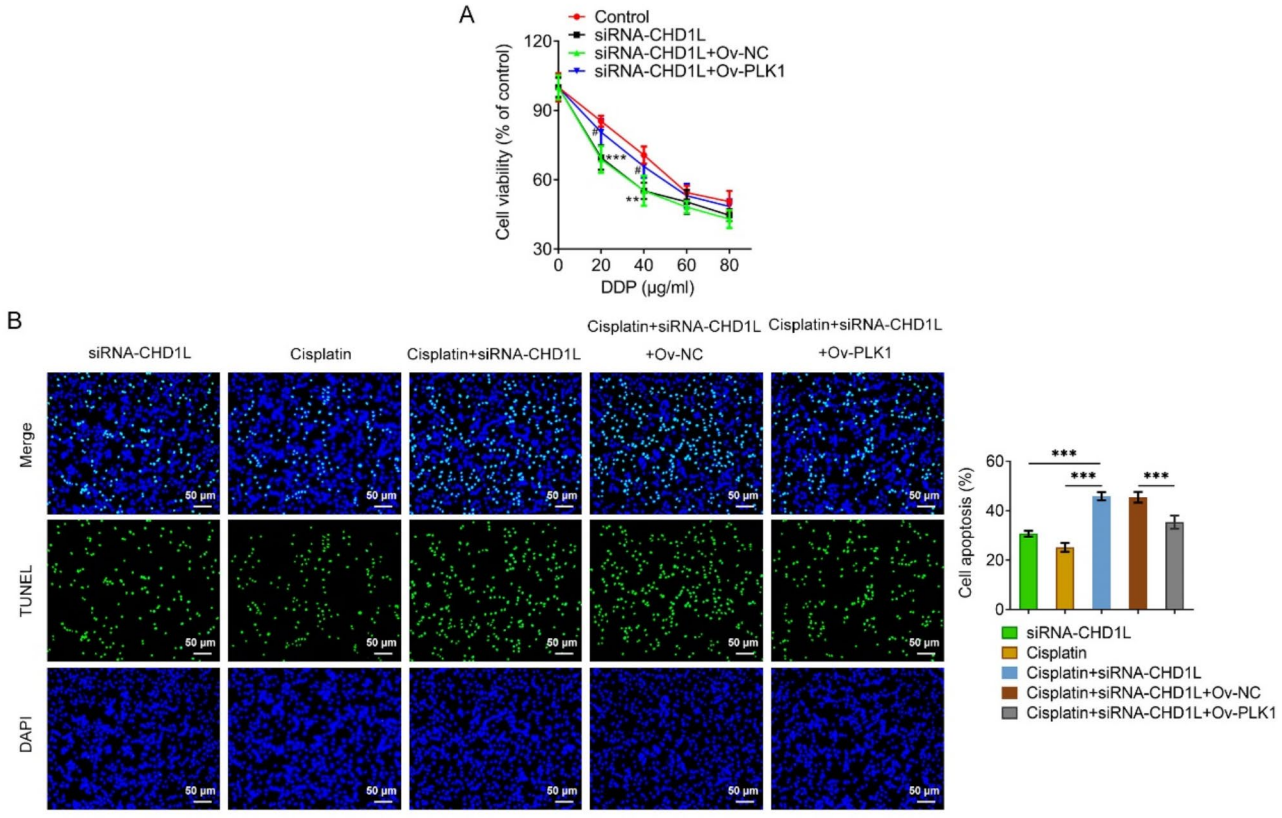

Fig. 8 Interference with CHD1L promoted cisplatin sensitivity of OC cells by binding PLK1. (**A**) CAOV-3 cells were treated with varying concentrations of cisplatin, and then CCK8 detected the viability of CAOV-3 cells co-transfected with Ov-PLK1 and siRNA-CHD1L. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$ vs. siRNA- CHD1L + Ov-NC. (**B**) TUNEL assay detected the apoptosis of CAOV-3 cells. $^{***}P < 0.001$

cancer cells may be related to the abnormal expression of PLK1. PLK1 downregulation can restore the chemotherapy sensitivity of gastric cancer cells and enhance the efficacy of cisplatin [26]. In our experiments, it was found that interference with CHD1L could promote the cisplatin sensitivity of OC cells through PLK1.

Conclusion

In conclusion, interference with CHD1L inhibits proliferation, promotes apoptosis, induces G2/M phase arrest, suppresses migration and invasion as well as enhances cisplatin sensitivity of OC cells by downregulating PLK1 expression. Our findings might contribute to a better understanding of CHD1L's role in OC, and establishes a theoretical basis for CHD1L as a potential therapeutic gene for the treatment of OC.

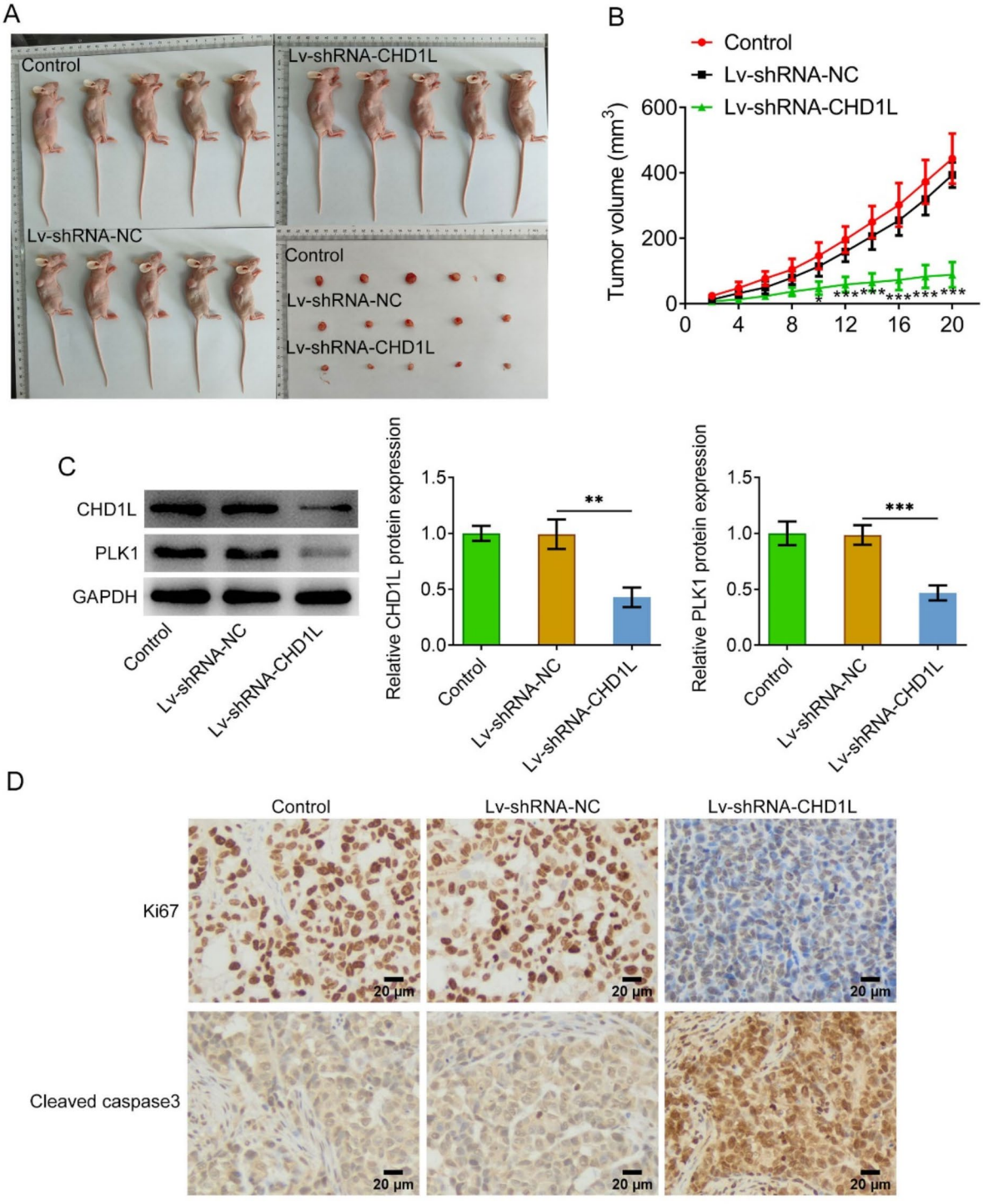

Fig. 9 CHD1L knockdown suppressed the development of subcutaneous CAOV-3 tumors in nude mice by downregulating PLK1 expression. (**A**) Images of tumor tissues in nude mice after transplantation. (**B**) Tumor volume in nude mice at different time was calculated. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. Lv-shRNA-NC. (**C**) Western blot was employed to analyze CHD1L and PLK1 expression in xenograft tumor tissues. (**D**) Ki67 and cleaved caspase3 expression was detected by immunohistochemical staining in xenograft tumor tissues. $^{**}P < 0.01$, $^{***}P < 0.001$

Author contributions
Kun Qiao conceived and designed the study. Kun Qiao, Yuanxiazi Guan and Wenjing Xing performed the experiments. Kun Qiao and Yuanxiazi Guan were major contributors to write the manuscript. All authors have read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
The animal experiments were approved by the Animal Ethics Committee of Shandong Provincial Maternal and Child Health Care Hospital.

Competing interests
The authors declare no competing interests.

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