

## Original Research

# Chrysin Synergistically Reverses Chemoresistance in Ovarian Cancer by Inhibiting Epithelial-Mesenchymal Transition

Nan Zhang<sup>1,2,†</sup>, Xiaoyuan Hao<sup>2,†</sup>, Chunying Zhuang<sup>3,4</sup>, Chenjing Zhu<sup>5</sup>, Sicong Liu<sup>1</sup>, Xiaoyuan Lu<sup>1</sup>, Yuxian Xie<sup>1</sup>, Ke'er Jiang<sup>1</sup>, Mengyu Li<sup>1</sup>, Jialin Zhu<sup>1</sup>, Ting Luo<sup>3,4</sup>, Xiaoxiao Liu<sup>6</sup>, Buze Chen<sup>1,\*</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou Medical University, 221000 Xuzhou, Jiangsu, China

<sup>2</sup>Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Xuzhou Medical University, 221000 Xuzhou, Jiangsu, China

<sup>3</sup>Institute of Breast Health Medicine, Breast Center, West China Hospital, Sichuan University, 610041 Chengdu, Sichuan, China

<sup>4</sup>Department of Medical Oncology, Cancer Center, West China Hospital, Sichuan University, 610041 Chengdu, Sichuan, China

<sup>5</sup>Department of Radiation Oncology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & Affiliated Cancer Hospital of Nanjing Medical University, 210009 Nanjing, Jiangsu, China

<sup>6</sup>Department of Radiation Oncology, Cancer Center, Affiliated Hospital of Xuzhou Medical University, 221000 Xuzhou, Jiangsu, China

\*Correspondence: [cbz001992@163.com](mailto:cbz001992@163.com) (Buze Chen)

†These authors contributed equally.

Academic Editor: Samir A. Farghaly

Submitted: 7 April 2026 Revised: 6 May 2026 Accepted: 22 May 2026 Published: 19 October 2026

## Abstract

**Background:** Ovarian cancer (OC), a lethal gynecological malignancy, frequently develops resistance to cisplatin, which significantly contributes to chemotherapy failure. Chrysin, a natural flavonoid, has exhibited anti-tumor and chemosensitizing effects, but evidence specific to OC remains limited. **Methods:** Parental SKOV3 and CAOV3 cells were treated with chrysin alone to assess cell viability (cell counting kit-8 (CCK-8) and colony formation assays) and apoptosis (flow cytometry). The synergistic effects of chrysin combined with cisplatin were evaluated using CCK-8 assays and CompuSyn 1.0 software (ComboSyn, Inc., Paramus, NJ, USA). Subsequently, two cisplatin-resistant sublines (SKOV3/DDP and CAOV3/DDP) were generated by gradually increasing cisplatin exposure. Changes in epithelial-mesenchymal transition (EMT) protein levels between parental and resistant cells were compared using Western blotting, followed by repeated viability (CCK-8), colony formation, Transwell migration, and apoptosis (flow cytometry) assays in resistant lines. Transcriptome sequencing was performed to identify differentially expressed genes. Additionally, EMT-related proteins (E-cadherin, N-cadherin, vimentin, and Snail1) and components of the nuclear factor kappa B (NF-κB) pathway (p-P65, P105/P50, p-IKKα/β, and p-IκBα) were analyzed by Western blotting. A rescue experiment using the NF-κB activator phorbol 12-myristate 13-acetate (PMA) confirmed the involvement of the NF-κB pathway. **Results:** Chrysin inhibited proliferation, induced apoptosis, and synergistically enhanced the efficacy of cisplatin in both parental and cisplatin-resistant OC cells. Mechanistically, chrysin suppressed the NF-κB pathway, consequently reversing EMT associated with cisplatin resistance. **Conclusions:** Chrysin synergistically enhances cisplatin efficacy against OC, including cisplatin-resistant cells, by modulating the NF-κB/EMT pathway. These findings suggest that chrysin could serve as a promising strategy for overcoming cisplatin resistance and merit clinical investigation.

**Keywords:** ovarian cancer; chrysin; chemoresistance; NF-κB; epithelial-mesenchymal transition

## 1. Introduction

Among female reproductive malignancies, ovarian cancer (OC) ranks third after cervical and endometrial cancers in incidence but leads in mortality [1]. Globally, the annual incidence of OC is approximately 324,398 cases, resulting in 206,839 deaths [2], and about 80% of patients initially present at an advanced stage. Among patients with advanced OC, the initial remission rate reaches approximately 80%; however, nearly three-quarters experience recurrence within the first two years post-treatment. The 5-year overall survival (OS) rate for OC patients is with advanced-stage OC is only approximately 30%, reflecting a very poor prognosis [3].

The standard initial treatment for OC has traditionally consisted of surgery combined with systemic chemotherapy. For patients across all stages (I–IV), global clinical guidelines recommend intravenous platinum-based chemotherapy as the standard first-line systemic treatment [4,5]. Approximately 85% of epithelial ovarian carcinoma patients experience recurrence following initial diagnosis and frontline platinum therapy, with recurrent disease typically becoming resistant to subsequent salvage treatments [6]. Thus, innovative combination therapies capable of overcoming chemoresistance and enhancing drug sensitivity represent promising strategies to prevent lethal recurrences in OC. Several natural products have been explored



for this purpose; for example, silymarin has demonstrated enhanced cytotoxicity of cisplatin (DDP) in OC cells [7]. Similarly, pyrogallol has been reported to increase OC cell sensitivity to cisplatin [8], and glycyrrhizic acid has exhibited synergistic tumor-suppressive activity with cisplatin in OC cells [9].

Chrysin (ChR) is a flavonoid compound derived from *Oroxylum indicum*, a species belonging to the Bignoniaceae family. Chemically identified as 5,7-dihydroxyflavone and also known as chrysine or koine, ChR exhibits a wide range of pharmacological activities. Accumulating evidence has demonstrated that ChR possesses multiple biological properties, including antioxidant, antitumor, antibacterial, and antidiabetic effects, and it is commercially marketed as a dietary supplement [10,11,12,13]. Moreover, ChR has been shown to have anticancer activity against various malignancies, including cervical cancer [14], bladder cancer [15], prostate cancer [16], and colorectal cancer [17]. Notably, ChR-induced suppression of the PI3K/AKT signaling pathway triggers ferroptosis in tumor cells, enhancing renal cancer cell sensitivity to sunitinib [18]. Although these findings underscore its potential in clinical combination therapies, research investigating the role of ChR in OC chemoresistance remains limited.

Our previous study reported synergistic anticancer activity when ChR was combined with pyrotinib against HER2-positive breast cancer [19]. Additionally, ChR was found to enhance tamoxifen efficacy in breast cancer cells, indicating broader potential as a chemosensitizer [20]. Thus, we hypothesized that it might exhibit a similar chemosensitizing effect in combination with DDP in cisplatin-resistant OC. The present study aimed to investigate whether ChR reverses cisplatin resistance in OC cells and to elucidate the underlying molecular mechanisms, with particular emphasis on epithelial-mesenchymal transition (EMT) and the NF- $\kappa$ B signaling pathway.

## 2. Materials and Methods

### 2.1 Reagents and Antibodies

Chrysin (ChR; cat. no. JOT-1005; Pufei, Chengdu, Sichuan, China) has the chemical formula  $C_{15}H_{10}O_4$ , a molecular weight of 254.24 g/mol, and a purity of 99.24%. Cisplatin (DDP; cat. no. HY-17394; MedChemExpress, Shanghai, China) was purchased from MedChemExpress. Phorbol 12-myristate 13-acetate (PMA; cat. no. GN10444; GIPBio, Shanghai, China) was acquired from GIPBio. The cell counting kit-8 (CCK-8; cat. no. VC5001L; VICMED, Xuzhou, Jiangsu, China) was used for cell viability assays. The antibodies used for Western blot (WB) analysis were as follows: E-cadherin (cat. no. 20874-1-AP; Proteintech, Wuhan, Hubei, China; 1:1000), N-cadherin (cat. no. 22018-1-AP; Proteintech, Wuhan, Hubei, China; 1:2000), Vimentin (cat. no. BF8006; Affinity Biosciences, Jiangsu, China; 1:1000), Snail1 (cat. no. 13099-1-AP; Proteintech, Wuhan, Hubei, China; 1:2000), Twist1 (cat. no. 25465-

1-AP; Proteintech, Wuhan, Hubei, China; 1:2000), p-I $\kappa$ B $\alpha$  (Ser32/36) (cat. no. WL02495; Wanleibio, Shenyang, Liaoning, China; 1:1000), p-IKK $\alpha$ / $\beta$  (cat. no. WLA0347; Wanleibio, Shenyang, Liaoning, China; 1:1000), P105/P50 (cat. no. WL01866; Wanleibio, Shenyang, Liaoning, China; 1:1000), p-P65 (cat. no. WL02469; Wanleibio, Shenyang, Liaoning, China; 1:1000), multidrug resistance protein 1 (MDR1)/P-glycoprotein (cat. no. WL02395; Wanleibio, Shenyang, Liaoning, China; 1:1000), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; cat. no. HRP-60004; Proteintech, Wuhan, Hubei, China; 1:10,000). HRP-conjugated goat anti-rabbit IgG (cat. no. RGAR001; Proteintech, Wuhan, Hubei, China) and HRP-conjugated goat anti-mouse IgG (cat. no. RGAM001; Proteintech, Wuhan, Hubei, China) as secondary antibodies.

### 2.2 Cell Culture

SKOV3 (human ovarian cancer cell line), CAOV3 (human ovarian adenocarcinoma cell line), and IOSE80 (human normal ovarian epithelial) cells were originally sourced from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). SKOV3 and CAOV3 were maintained in Dulbecco's modified Eagle medium (cat. no. KGL1206, Kaiji, Nanjing, Jiangsu, China) with 10% fetal bovine serum (Vazyme, China, F101-01), while IOSE80 was cultured in RPMI 1640 medium (cat. no. KGL1501-500, Kaiji, Nanjing, Jiangsu, China) with 10% fetal bovine serum (cat. no. F101-01, Vazyme, Nanjing, Jiangsu, China). To maintain all cell lines, we used a 5% CO<sub>2</sub> humidified atmosphere and a temperature of 37 °C. All cell lines have been authenticated by short tandem repeat (STR) analysis. Routine mycoplasma detection was performed before experiments, and all cells were confirmed to be negative for mycoplasma contamination to ensure the quality of cells used in the study.

Cisplatin-resistant sublines were established by continuously exposing SKOV3 and CAOV3 to gradually increasing cisplatin concentrations. The initial cisplatin concentration was 1  $\mu$ g/mL and was gradually increased to 15  $\mu$ g/mL throughout a 9-month period. At each step, cells were maintained at a given concentration for at least two passages before the concentration was increased, provided that cell growth returned to normal. After reaching the final concentration (15  $\mu$ g/mL), the resistant cells (designated SKOV3/DDP and CAOV3/DDP, i.e., the cisplatin-resistant SKOV3 and CAOV3 cell lines, respectively) were grown in culture medium containing 2  $\mu$ g/mL cisplatin to sustain the resistant phenotype.

### 2.3 Human Tissues and Immunohistochemistry (IHC)

Human OC tissues and non-tumor tissues were procured from the Affiliated Hospital of Xuzhou Medical University between June 2025 and November 2025. This research was carried out in accordance with government regulations and the Declaration of Helsinki. Human tissue IHC

staining was done by Wuhan Servicebio Technology Co., Ltd. (Wuhan, Hubei, China). The tissues were fixed in 10% paraformaldehyde (cat. no. VIH100; VICMED, Xuzhou, Jiangsu, China). 4- $\mu$ m thick sections were cut from the resulting paraffin blocks. After antigen retrieval, the primary antibody was applied to the sections, which were then kept at 4 °C overnight. The next day, they were exposed to a secondary antibody, then stained with chromogen and hematoxylin, and finally viewed under a light microscope. Two independent pathologists scored the IHC results using the H-score method, as described previously [21].

## 2.4 Cell Viability Assays

Viability of both drug-treated and control OC cells was quantified by CCK-8 (cat. no. VC5001L; VICMED, Xuzhou, Jiangsu, China) colorimetry. Briefly, cells were plated at a density of  $5 \times 10^3$  cells per well in 96-well plates, followed by a 24-hour incubation to allow cell adhesion. To assess drug effects, cells were incubated with the specified concentrations for 24, 48, or 72 h. After this exposure, CCK-8 reagent was introduced into each well. The mixture was further kept for 2 h under the conditions of 37 °C in a 5% CO<sub>2</sub> atmosphere in the dark. Absorbance at 450 nm was subsequently determined with a microplate reader (YOMim UReader-Eyes, Hangzhou, Zhejiang, China).

## 2.5 Colony Formation Assays

Each well of the 6-well plates received 1000 cells, which were then cultured at 37 °C for 10 days. Culture ended when colonies contained at least 50 cells. Following medium aspiration, cultures were rinsed three times with PBS, subjected to fixation with 4% paraformaldehyde for 10 min, and counterstained with 0.1% crystal violet (cat. no. G1014-50ML; Servicebio, Wuhan, Hubei, China) for 15 min. Following mild rinsing and natural drying at room temperature, microscopic imaging was performed, and colony numbers were quantified with ImageJ 1.52v software (National Institutes of Health, Bethesda, MD, USA).

## 2.6 Flow Cytometric Analysis of Apoptosis

$2 \times 10^5$  cells/well were inoculated into 6-well plates and maintained overnight under standard conditions. Cells were exposed to the indicated drug concentrations for 48 h, digested with EDTA-free trypsin (cat. no. C100C1; NCM Biotech, Suzhou, Jiangsu, China), and labeled using Annexin V-FITC/PI Apoptosis Detection Kit (cat. no. KGA1102; Kaiji, Jiangsu, China) following the manufacturer's protocol. A flow cytometer (BD LSRFortessa; BD Biosciences, San Jose, CA, USA) was employed for sample analysis. Early apoptotic cells were defined as Annexin V-FITC-positive/PI-negative, late apoptotic cells as Annexin V-FITC-positive/PI-positive, and total apoptosis was calculated as the sum of early and late apoptotic cells.

## 2.7 CompuSyn Analysis

To evaluate synergistic, additive, or antagonistic interactions between ChR and DDP, CompuSyn 1.0 was used to calculate the combination index (CI). Dose-effect curves, CI plots, and isobolograms were generated. Data from the cell viability assays were used for CompuSyn analysis [22,23,24]. The core principle of CompuSyn lies in calculating the CI for each drug combination, which serves as a quantitative measure to assess the strength of drug-drug interactions at a specified effect level (e.g., fraction of cells affected). In essence, a combination index below unity implies synergistic action, unity itself signifies additivity, and values exceeding unity denote antagonism.

## 2.8 WB Analysis

After treatment with different concentrations of ChR or DDP, total cellular protein was extracted using RIPA lysis buffer (cat. no. P0013; Beyotime, Shanghai, China) supplemented with protease inhibitors and phosphatase inhibitors. Protein concentrations were quantified with a BCA kit (cat. no. KGB2101-5000; Kaiji, Nanjing, Jiangsu, China). Equivalent amounts of protein underwent SDS-PAGE and were then transferred to PVDF membranes (cat. no. ISEQ00010; Millipore, Burlington, MA, USA). The membranes were blocked for 1 hour at room temperature with 5% skim milk. The membranes were incubated with primary antibodies overnight at 4 °C. Afterward, unbound antibodies were removed by washing with TBST. The samples were subsequently probed with secondary antibodies for 1 h at room temperature. ECL substrate (cat. no. KGC4602-500; Kaiji, Nanjing, Jiangsu, China) was employed to detect target protein signals, which were then acquired using a Bio-Rad documentation system. ImageJ software was used to process and analyze the protein gray value.

## 2.9 Migration and Invasion Assays

Transwell chambers were pre-coated with Matrigel (cat. no. HY-K6001; MedChemExpress, Shanghai, China) diluted 1:5 in serum-free medium, then kept at 37 °C for 5 h for invasion assays. Cells in log phase were collected. The final working concentration was set at  $2 \times 10^5$  cells/mL by diluting the cells in basal medium devoid of serum. 200  $\mu$ L of cell suspension was added to the upper chamber for both migration and invasion assays. For the lower chamber, 600  $\mu$ L of complete medium with different drug concentrations was added. Migration assays ran for 24 hours, while invasion assays ran for 48 hours. After that, fixation was done with 4% paraformaldehyde for 15 min, followed by three PBS washes, a 15-min crystal violet stain, and a final rinse with distilled water. The non-migrated cells remaining on the upper membrane surface were gently wiped off with a cotton swab. Then, the samples were photographed under a microscope.

### 2.10 Transcriptomic Profiling by RNA-Seq

Total RNA was isolated using TRIzol® reagent (cat. no. 15596026CN; Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions; RNA-Seq library construction and sequencing were performed by Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China). RNA integrity was assessed via Agilent 5300 Bioanalyzer analysis (Agilent Technologies, Santa Clara, CA, USA), and concentrations were determined by NanoDrop 2000 spectrophotometry (Thermo Fisher Scientific, Waltham, MA, USA). All procedures from RNA processing to sequencing adhered to Illumina standard protocols (San Diego, CA, USA). The RNA-Seq transcriptome library was constructed using the Illumina® Stranded mRNA Prep Ligation kit with 1 µg of total RNA. Briefly, mRNA was isolated using oligo(dT) beads based on polyA selection, then fragmented with the fragmentation buffer. Double-stranded cDNA was generated using the SuperScript system (Invitrogen, CA) with random hexamer primers (Illumina). The resulting products subsequently underwent end-repair, phosphorylation, and A-tailing per the Illumina library construction protocol. Fragments of ~300 bp were enriched by gel extraction on 2% Low Range Ultra Agarose and subjected to 15 cycles of PCR using Phusion DNA polymerase (New England Biolabs, Ipswich, MA, USA). The libraries were finally sequenced with 150 bp paired-end reads. Raw sequencing data were processed using FastQC for quality control, and adapter sequences and low-quality reads were removed using Trimmomatic.

Differential expression analysis (DEGs) between drug-resistant sublines (SKOV3/DDP and CAOV3/DDP) and their drug-naïve counterparts (SKOV3 and CAOV3) was conducted using DESeq2 (version 1.48.1), with significant DEGs defined as  $|\log_2FC| \geq 1$  and false discovery rate (FDR)  $\leq 0.05$ . Upregulated DEGs ( $\log_2FC \geq 1$ ) and downregulated DEGs ( $\log_2FC \leq -1$ ) were analyzed separately. Pathway enrichment analysis was primarily performed on upregulated DEGs to identify activated signaling pathways associated with cisplatin resistance, unless otherwise specified. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment elucidated functional implications. Comprehensive gene sets are documented in **Supplementary Table 1**; raw signatures remain accessible to qualified researchers upon solicitation.

### 2.11 Survival Analysis

Survival information was retrieved from the Kaplan-Meier Plotter database (<https://www.kmplot.com>), which compiles gene expression and clinical datasets from public databases such as Gene Expression Omnibus (GEO), European Genome-phenome Archive (EGA), and The Cancer Genome Atlas (TCGA). Overall survival (OS) was calculated from the time of initial diagnosis to the time of all-cause death. Progression-free survival (PFS) was measured from diagnosis until the earliest occurrence of disease pro-

gression or all-cause mortality. In the KM Plotter database, PFS data are not available for all patients due to incomplete imaging follow-up; therefore, the evaluable sample size for PFS ( $n = 1435$ ) is smaller than that for OS. The median expression of the target gene was used to classify patients. Survival curves were constructed using the KM Plotter web interface, and between-group differences were assessed with the log-rank test.

### 2.12 Statistical Analysis

Every assay was executed in at least three independent replicates. We analyzed the data statistically in GraphPad Prism (v10.1.2; GraphPad Software, Boston, MA, USA) and R (v4.2.2; R Foundation for Statistical Computing, Vienna, Austria). Dichotomous comparisons utilized unpaired two-tailed Student's *t*-tests; for polychotomous datasets, one-way ANOVA was implemented with subsequent Tukey's HSD adjustment. Significance was declared at  $p < 0.05$ .

## 3. Results

### 3.1 ChR Suppresses the Proliferation of SKOV3 and CAOV3 OC Cells

ChR is a flavonoid compound with a well-defined chemical structure, as illustrated in Fig. 1A. To elucidate how ChR influences OC cell behavior, we performed CCK-8 assays to assess its effect on the viability of SKOV3 and CAOV3 cells. We found that the inhibition of OC cell viability escalated as ChR concentration increased and treatment time lengthened, indicating that ChR exerts an anti-viability effect on OC cells (Fig. 1B,C).

Half-maximal inhibitory concentration ( $IC_{50}$ ) were determined for ChR against SKOV3 and CAOV3 cells across a 24–72 h time course.  $IC_{50}$  estimates at individual time points are provided in **Supplementary Table 2**. Notably, prolonged exposure reduced ChR  $IC_{50}$  values, indicating time-dependent potency enhancement. ChR inhibited cell viability in both cell lines. Furthermore, the results shown in Fig. 1D indicated that the clonogenic capacity of OC cells was gradually reduced with increasing ChR concentrations.

Apoptosis assays were performed to determine whether ChR exerts a proapoptotic effect on OC cells and to further elucidate the mechanisms underlying its anticancer activity. We simultaneously labeled CAOV3 cells with Annexin V-FITC and PI and quantified apoptotic fractions on a BD Fortessa platform. The levels of apoptotic cells were highest in the group cultured with 60 µmol/L ChR (Fig. 1E). These findings demonstrate that ChR treatment promotes apoptosis in OC cells, supporting the notion that apoptosis induction may be one of the mechanisms underlying its anticancer efficacy.

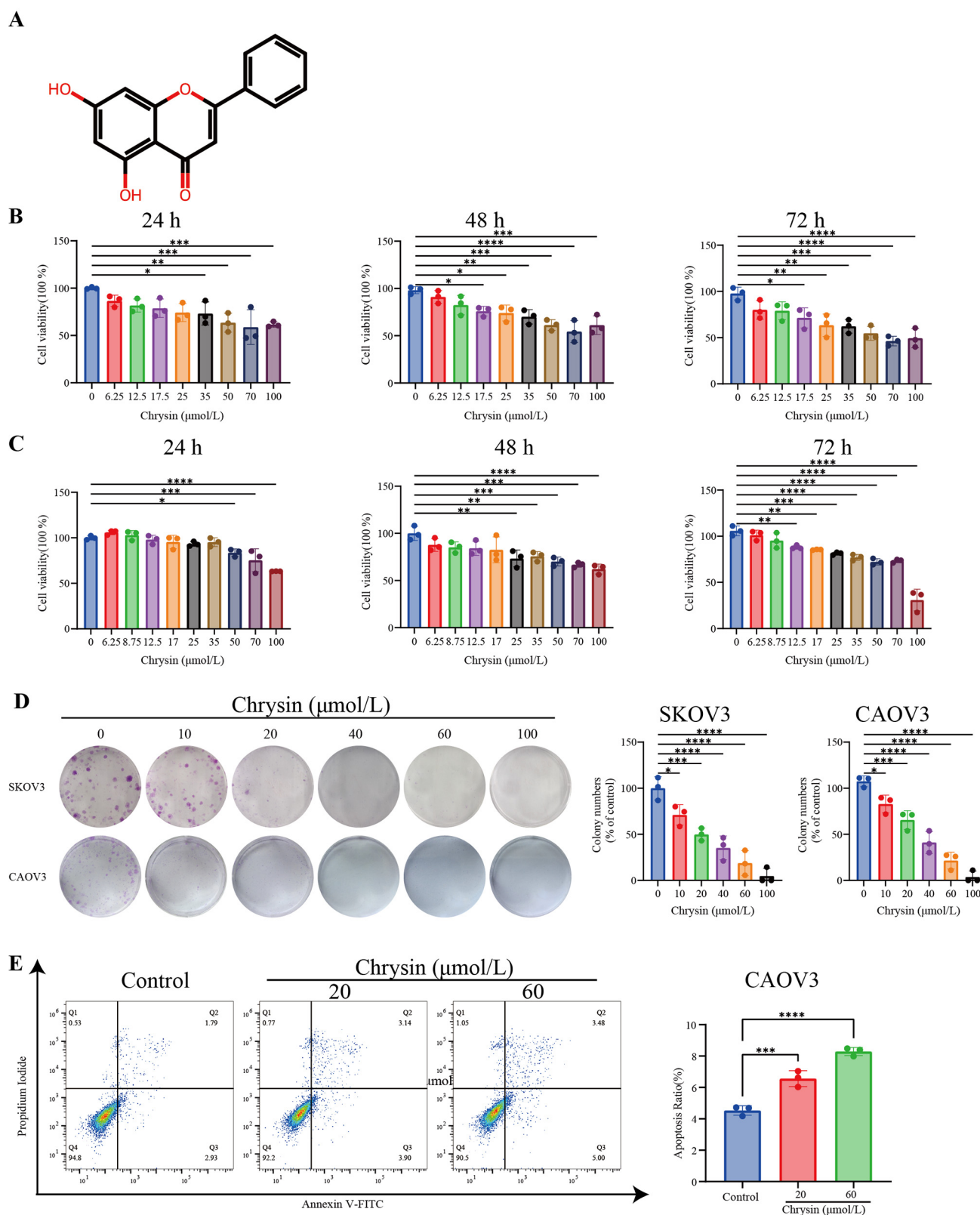


**Fig. 1. ChR represses proliferation and promotes apoptosis in OC cells.** (A) The molecular architecture of ChR. (B) SKOV3 and (C) CAOV3 cells were challenged with escalating ChR doses for 24, 48, and 72 h prior to the CCK-8 assay. (D) Clonogenic potential was evaluated by colony formation assay. (E) Apoptotic fractions in CAOV3 cultures following ChR challenge were determined by flow cytometry.  $n = 3$ ,  $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ ;  $****p < 0.0001$ . ChR, chrysin; OC, ovarian cancer; SKOV3, human ovarian cancer cell line; CAOV3, human ovarian adenocarcinoma cell line; CCK-8, cell counting kit-8.

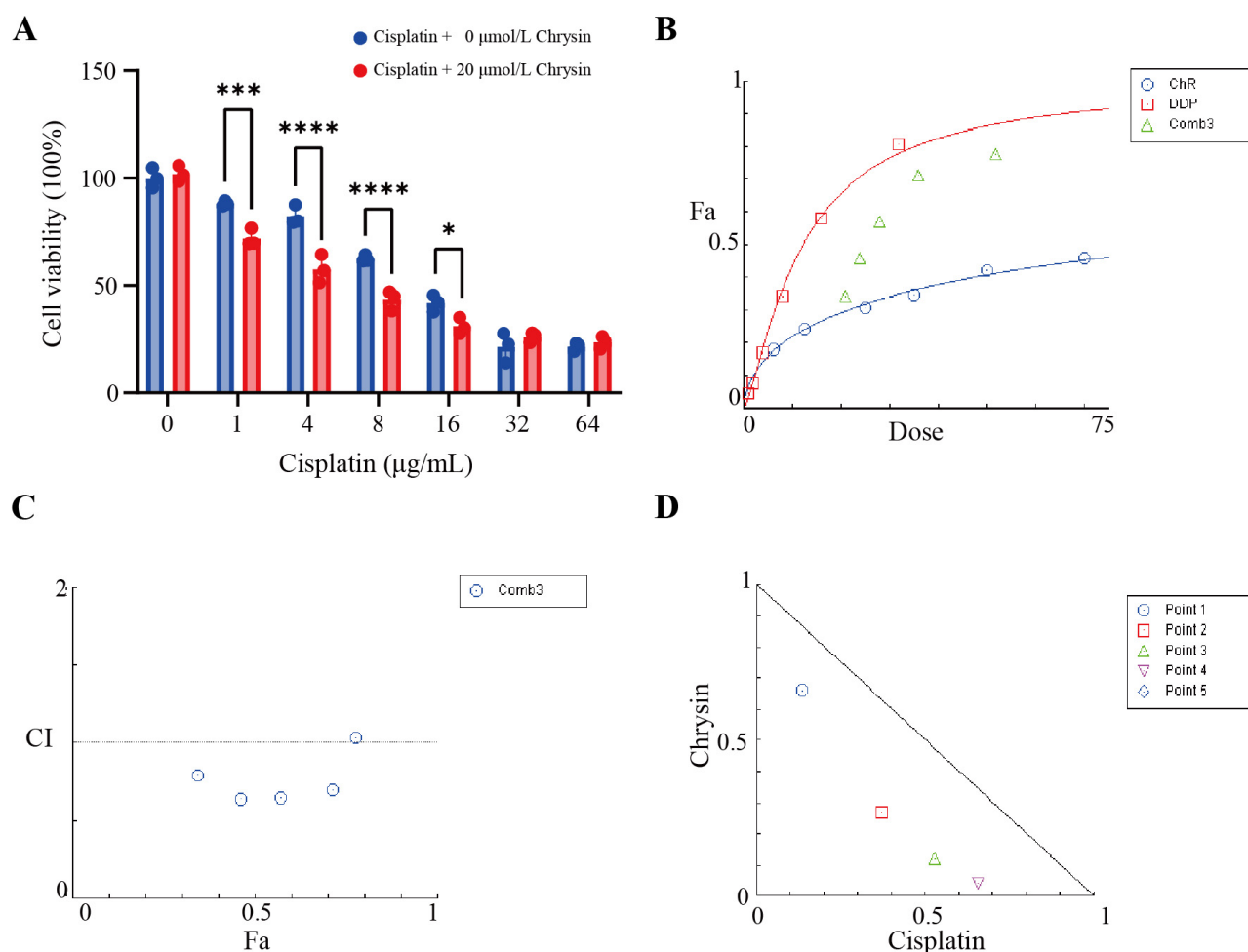


**Fig. 2. Effects of ChR and DDP, either individually or in combination, on SKOV3 cell viability.** (A) SKOV3 cells were exposed to DDP alone or in combination with 20 μmol/L ChR for 48 h prior to the CCK-8 assay. (B–D) Synergy parameters generated by CompuSyn: (B) dose–effect curves, (C) combination index (CI) plots, and (D) isobolograms. Comb3: DDP combined with 20 μmol/L ChR.  $n = 3$ ,  $*p < 0.05$ ;  $***p < 0.001$ ;  $****p < 0.0001$ . DDP, cisplatin; Fa, Fraction of cells affected by combination therapy.

### 3.2 ChR and DDP in Combination Suppress the Proliferation of Ovarian Cancer Cells

When we treated SKOV3 cells with ChR together with DDP, the combination caused a clear drop in viability. As shown in Fig. 2A, co-treatment with 20 μmol/L ChR and gradient concentrations of DDP for 48 h significantly enhanced the inhibition of SKOV3 cell viability.

CompuSyn analysis revealed a dose-dependent inhibitory pattern for ChR monotherapy, DDP monotherapy, and their combination, with inhibition rates increasing as drug doses increased (Fig. 2B). Combination index plots and isobolograms further verified the synergistic inhibitory effect of the co-treatment (Fig. 2C,D). Furthermore, we investigated the antiproliferative activity in SKOV3 cells treated with 10, 15, or 25 μmol/L ChR in combination with different concentrations of DDP (Supplementary Fig. 1). The corresponding dose-effect curves, combination index plots, and isobolograms all demonstrated limited synergistic enhancement at these ChR concentrations.

Collectively, these findings confirm that ChR acts synergistically with DDP to inhibit SKOV3 cell proliferation, with the combination containing 20 μmol/L ChR exerting the most pronounced effect.

### 3.3 ChR and DDP Synergistically Inhibit the Proliferation of OC Cells

To gain insight into the synergistic potential of the ChR-DDP combination, we used CompuSyn software (version 1.0) to characterize their synergistic or antagonistic effects. CompuSyn is widely used to calculate the CI, where  $CI < 1$  indicates synergism,  $CI = 1$  indicates an additive effect, and  $CI > 1$  indicates antagonism. We summarize the combined effects of ChR and DDP on SKOV3 cells in Table 1. Synergism was observed in most ChR-DDP combinations. The strongest synergistic effect was observed at 20 μmol/L ChR combined with 8 μg/mL DDP ( $CI = 0.5557$ ).

**Table 1. Fa and CI data for ChR-DDP combinations in SKOV3 cells.**

| Cisplatin<br>( $\mu\text{g/mL}$ ) | Chrysin<br>( $\mu\text{mol/L}$ ) | Fa     | CI     | Conclusion |
|-----------------------------------|----------------------------------|--------|--------|------------|
| 4                                 | 10                               | 0.3680 | 0.7715 | Synergism  |
| 4                                 | 15                               | 0.3458 | 1.0221 | Antagonism |
| 4                                 | 20                               | 0.4801 | 0.5884 | Synergism  |
| 4                                 | 25                               | 0.3632 | 1.2129 | Antagonism |
| 4                                 | 30                               | 0.4626 | 0.7743 | Synergism  |
| 8                                 | 10                               | 0.5421 | 0.6567 | Synergism  |
| 8                                 | 15                               | 0.4399 | 1.0326 | Antagonism |
| 8                                 | 20                               | 0.6126 | 0.5557 | Synergism  |
| 8                                 | 25                               | 0.4997 | 0.9190 | Synergism  |
| 8                                 | 30                               | 0.5748 | 0.7041 | Synergism  |
| 16                                | 10                               | 0.6482 | 0.8624 | Synergism  |
| 16                                | 15                               | 0.5809 | 1.1150 | Antagonism |
| 16                                | 20                               | 0.7192 | 0.6816 | Synergism  |
| 16                                | 25                               | 0.6579 | 0.8808 | Synergism  |
| 16                                | 30                               | 0.7156 | 0.7112 | Synergism  |

Fa, Fraction of cells affected by combination therapy; CI, Combination index.

### 3.4 Cisplatin-Resistant Cell Lines Are Established, and Resistance-Associated Proteins Are Expressed

We established SKOV3/DDP and CAOV3/DDP by gradually raising DDP concentrations in the culture medium until the cells stably tolerated doses up to 15  $\mu\text{g/mL}$ . To validate the cisplatin-resistant phenotype of SKOV3/DDP and CAOV3/DDP cells, CCK-8 assays were performed. Cell viability curves clearly distinguished the parental cells from their resistant sublines (Fig. 3A–D). The resistance index (RI), calculated from the  $\text{IC}_{50}$  values, is provided in **Supplementary Table 3**. SKOV3/DDP cells exhibited a substantially higher RI of 8.14 compared with the CAOV3/DDP cells, which showed an RI of 4.45.

A large body of evidence has shown that upregulation of multidrug resistance protein 1 (MDR1) commonly decreases the intracellular accumulation of chemotherapeutic agents in tumor cells, thereby driving the development of drug resistance. Moreover, multiple research findings have confirmed abnormal MDR1 overexpression in cisplatin-resistant cancer cell lines [25,26,27,28]. Therefore, WB analysis was performed to detect MDR1 protein expression (Fig. 3E, **Supplementary Fig. 2A,B**), affirming the stable chemoresistant phenotype of these DDP-adapted OC cell lines.

As reported in the literature [29,30,31,32,33,34], EMT serves as a pivotal regulator of chemoresistance. We subsequently examined the expression profiles of EMT-related proteins in both parental and resistant cell lines. Our data demonstrated that, compared with their parental counterparts, the cisplatin-resistant cells exhibited marked upregulation of the mesenchymal markers N-cadherin, vimentin, and Snail1, accompanied by downregulation of the ep-

ithelial marker E-cadherin (Fig. 3F, **Supplementary Fig. 2C,D**).

### 3.5 ChR Overcame Chemotherapy Resistance in OC Cells

Our experimental data demonstrated that ChR treatment, particularly when combined with DDP, markedly impaired SKOV3/DDP and CAOV3/DDP cell viability (Fig. 4A,B). Meanwhile, ChR substantially suppressed the colony-forming capacity of these cells, and the combination showed enhanced inhibitory effects (Fig. 4C–E). In addition, ChR potently inhibited the invasive and migratory capabilities of the cells *in vitro*, the combination regimen demonstrated significantly enhanced efficacy compared with single-agent treatments in the monotherapy group (Fig. 4F–I). Flow cytometry also showed that ChR triggered apoptosis of cancer cells. The pro-apoptotic activity was further augmented when ChR was combined with DDP (Fig. 4J). Although combination treatment induced only a moderate increase in apoptosis, the marked suppression of cell proliferation and colony formation suggests the involvement of other, as yet unclear, mechanisms in the chemosensitizing activity of ChR.

### 3.6 ChR Inhibits EMT Progression in Cisplatin-Resistant OC Cells

To investigate whether ChR inhibits metastasis of cisplatin-resistant OC cells by reversing EMT, total RNA was extracted to construct sequencing libraries and perform RNA-Seq analysis. Comparative analysis between parental and cisplatin-resistant cells identified distinct transcriptional profiles. We identified DEGs that were upregulated in resistant cells when contrasted with their parental counterparts, i.e., low in parental cells and high in resistant cells. Pathway enrichment analysis was then performed on these DEGs to reveal signaling pathways linked to cisplatin resistance. Overexpressed transcript sets exhibited prominent enrichment within EMT, bladder cancer, and pathogenic *Escherichia coli* infection modules (Fig. 5A). Concordantly, KEGG pathway mapping demonstrated that elevated transcripts in DDP-resistant cells converged on EMT, focal adhesion, and TGF- $\beta$  signaling axes (Fig. 5B).

We next examined whether ChR could reverse these EMT-related changes. Western blot analysis was employed to assess EMT-related proteins in cisplatin-resistant OC cells. As shown in Fig. 5C and **Supplementary Fig. 2E** for SKOV3/DDP cells, Chrysin monotherapy exerted no significant effects on the expression levels of N-cadherin, E-cadherin, Vimentin and Snail1. DDP alone upregulated E-cadherin and downregulated N-cadherin, Vimentin, and Snail1. The ChR + DDP combination produced the strongest EMT reversal: increased E-cadherin (vs. NC, ChR, and DDP) and suppressed N-cadherin, Vimentin, and Snail1.

In CAOV3/DDP cells (Fig. 5D, **Supplementary Fig. 2F**), ChR alone similarly had no significant effect on N-

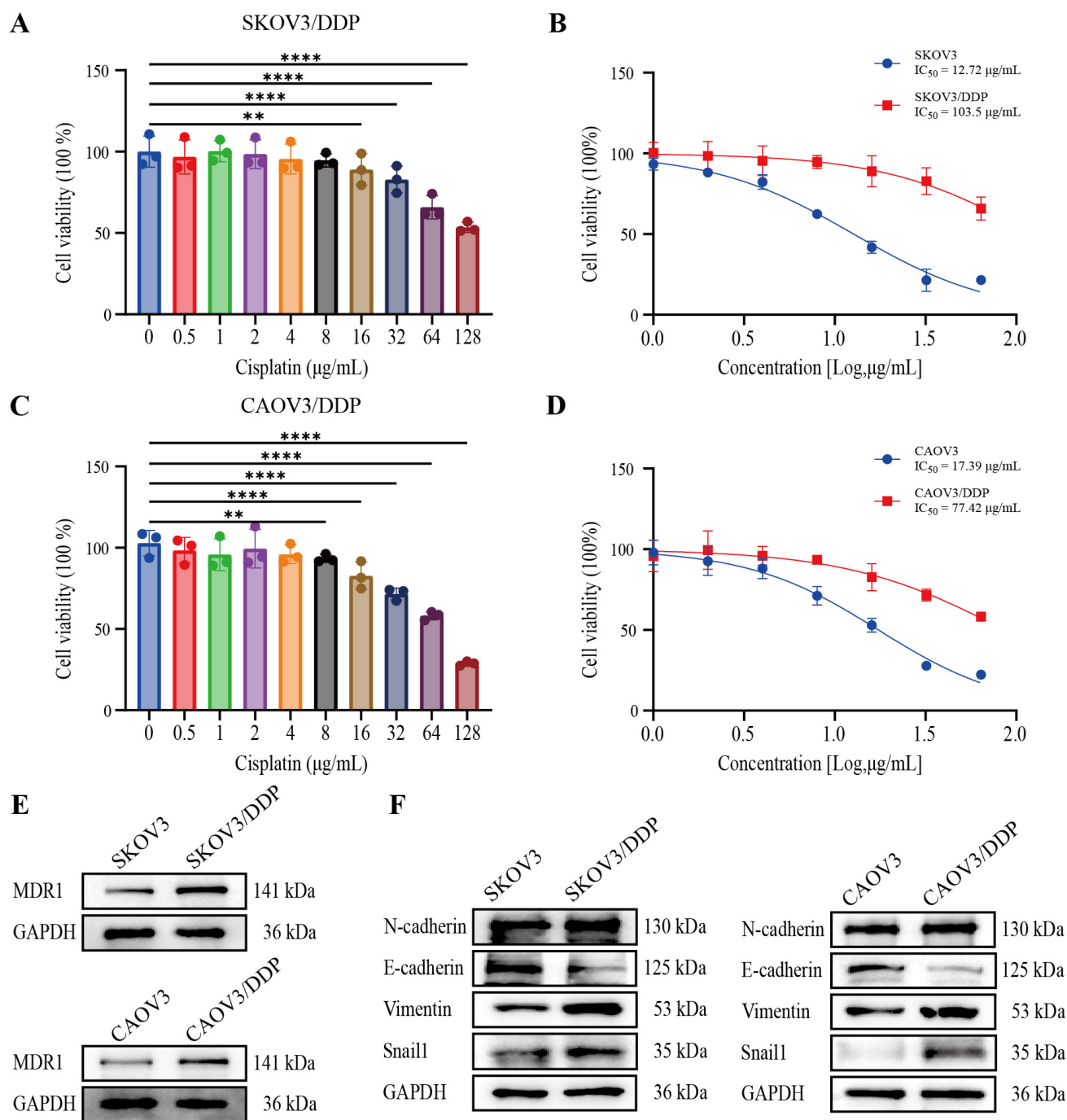


**Fig. 3. Establishment of cisplatin-refractory SKOV3/DDP and CAOV3/DDP sublines alongside EMT marker profiling.** (A–D) Cell viability of parental and cisplatin-resistant cell lines as determined by CCK-8 assay. (E) Immunoblot detection of MDR1/P-glycoprotein in SKOV3/CAOV3 parental and cisplatin-resistant counterparts. (F) EMT-associated protein expression (epithelial and mesenchymal markers) in naive versus resistant OC cell lines by WB.  $n = 3$ ,  $**p < 0.01$ ;  $****p < 0.0001$ . IC<sub>50</sub>, half-maximal inhibitory concentration; MDR1, multidrug resistance protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SKOV3/DDP, cisplatin-resistant SKOV3 cell line; CAOV3/DDP, cisplatin-resistant CAOV3 cell line; EMT, epithelial-mesenchymal transition; WB, Western blot.

cadherin, E-cadherin, Vimentin and Snail1. DDP alone up-regulated E-cadherin and down-regulated N-cadherin, Vimentin and Snail1 significantly. The combination again strongly reversed EMT: E-cadherin up-regulation (vs. NC,

ChR, and DDP) and N-cadherin/Vimentin/Snail1 suppression. Together, these findings indicate that ChR augmented DDP-mediated EMT suppression in cisplatin-resistant OC cells.

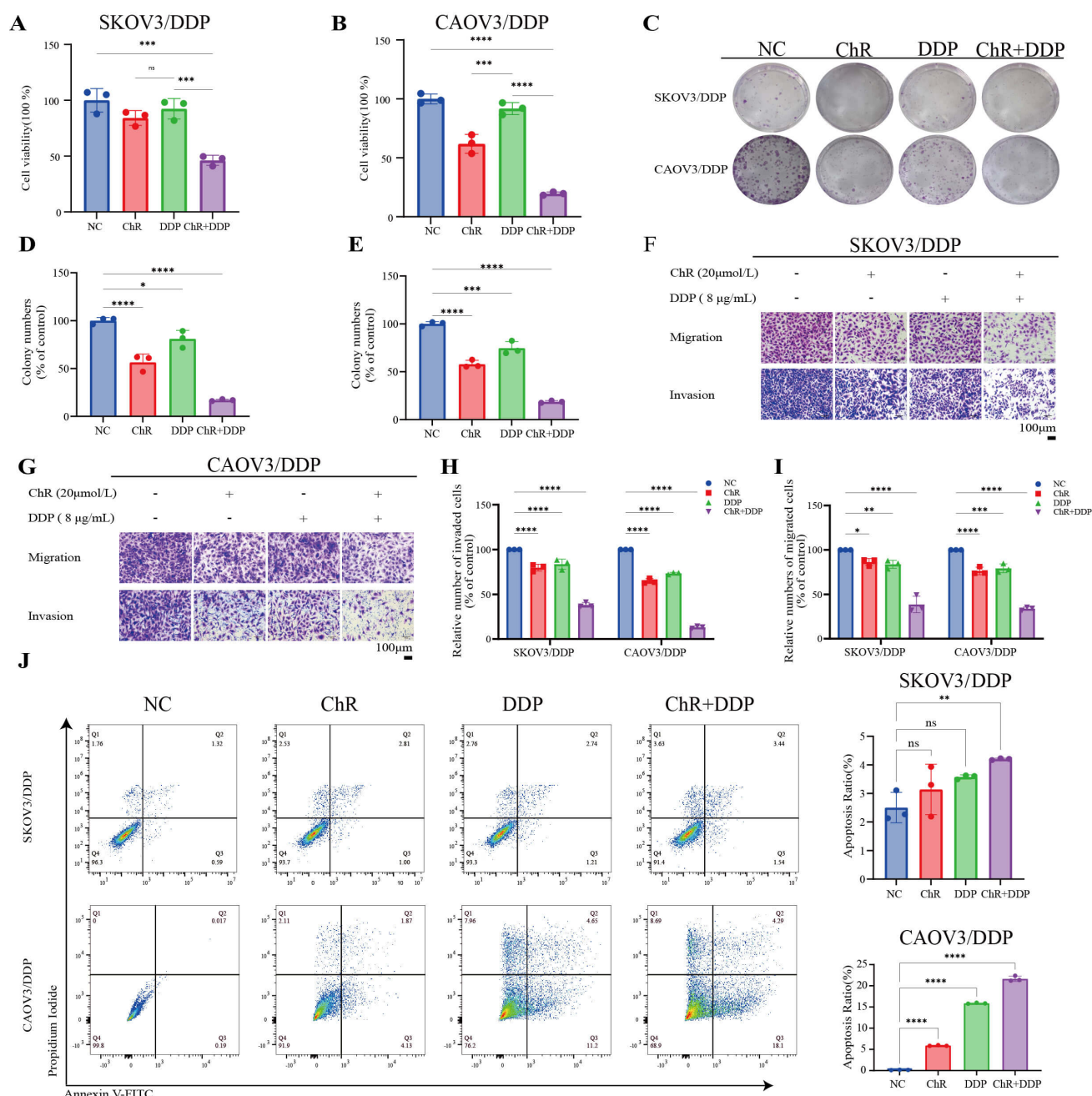


**Fig. 4. ChR overcomes chemotherapy resistance in OC cells.** (A,B) Viability of SKOV3/DDP and CAOV3/DDP cells after ChR, DDP, or their combination, measured by CCK-8 assays. (C–E) Colony formation of resistant cells treated under the same drug conditions. (F–I) Migration and invasion of drug-exposed SKOV3/DDP and CAOV3/DDP cells assessed by Transwell assays. Scale bar = 100  $\mu$ m. (J) Apoptosis rates in resistant cells treated with ChR alone or ChR + DDP, determined by flow cytometry.  $n = 3$ , \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ; ns, not significant; NC, negative control.

Twist1 is an important transcription factor in EMT. We first detected Twist1 expression in IOSE80 and OC cell lines and observed that Twist1 was significantly upregulated in cancer cells (Fig. 6A). In the cisplatin-resistant sublines, Twist1 expression was further elevated compared with their parental counterparts, suggesting an association between Twist1 upregulation and cisplatin resistance (Fig. 6B). Patient tissue samples supported that Twist1 expres-

sion was markedly elevated in OC tissues relative to non-malignant tissues (Fig. 6C,D). Elevated Twist1 expression predicted inferior OS and PFS in OC patients (Kaplan-Meier analysis, Fig. 6E), suggesting its potential as an unfavorable prognostic biomarker. Furthermore, ChR, as a single agent or together with DDP, reduced Twist1 expression in cisplatin-resistant OC cells. The ChR + DDP combination exerted superior Twist1-suppressive effects compared

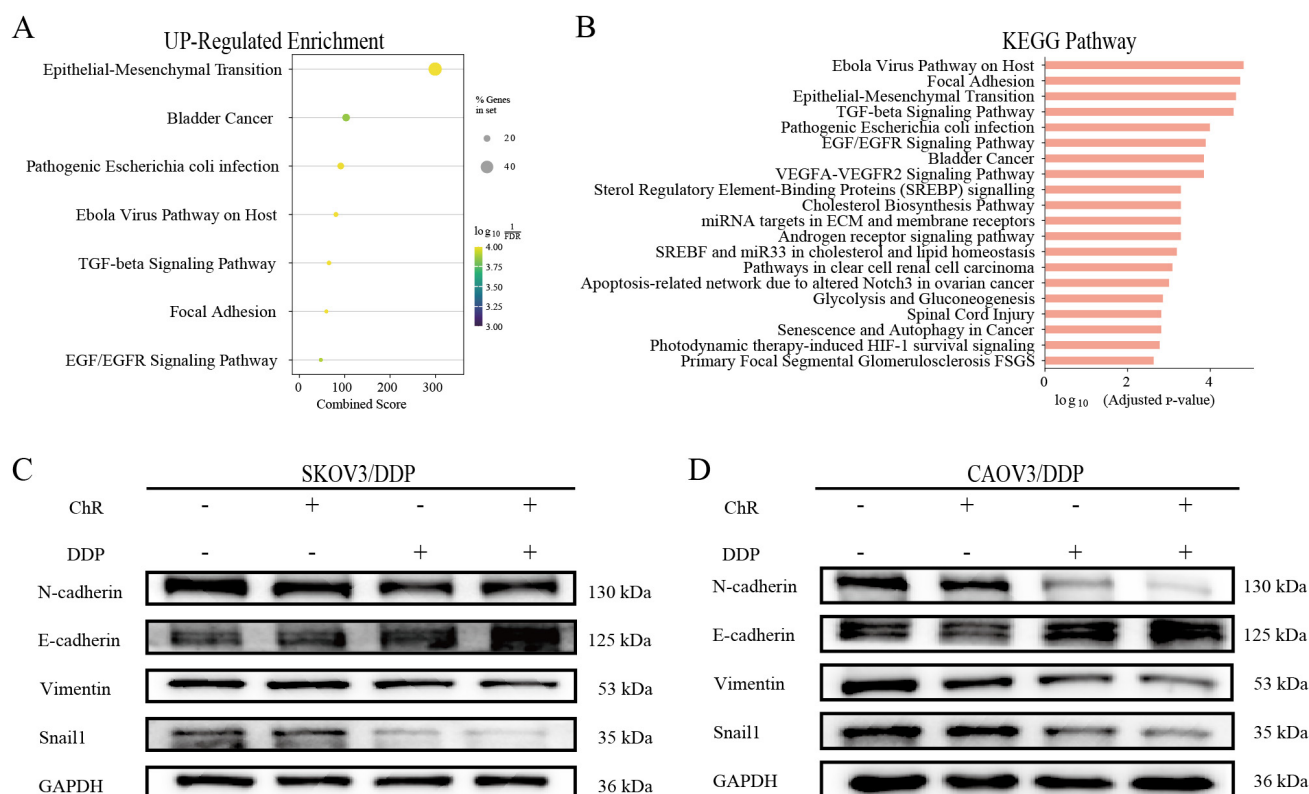


**Fig. 5. ChR reverses DDP resistance in OC by inhibiting EMT.** (A) Enriched pathways analysis of the different genes. (B) KEGG pathway enrichment analysis of the genes ranked by adjusted *p*-values. (C) WB analysis of EMT-related protein expression in SKOV3/DDP cells. (D) WB analysis of EMT-related protein expression in CAOV3/DDP cells. *n* = 3. KEGG, Kyoto Encyclopedia of Genes and Genomes.

with either monotherapy (Fig. 6F,G). These findings suggest that ChR may attenuate DDP resistance and metastatic potential in OC cells by suppressing Twist1-mediated EMT, with further enhanced efficacy upon co-administration with DDP.

### 3.7 ChR Reverses DDP Resistance by Suppressing the NF-κB/Twist1 Signaling Pathway

Numerous studies have demonstrated that ChR exerts its biological effects through modulation of the NF-κB signaling pathway [35,36,37,38,39]. To investigate the effects of ChR and DDP on NF-κB signaling in cisplatin-resistant OC cells, key pathway-related proteins were examined by WB analysis. In SKOV3/DDP cells (Fig. 7A and **Supplementary Fig. 2G**), neither ChR nor DDP alone altered the levels of P105/P50, p-IKKα/β or p-P65 compared with the NC group, while DDP monotherapy only slightly reduced p-IκBα. In contrast, the combination of ChR and DDP markedly downregulated all four NF-κB-related proteins, exhibiting significantly stronger inhibitory effects than either monotherapy.

Similar results were observed in CAOV3/DDP cells (Fig. 7B and **Supplementary Fig. 2H**). ChR alone exerted no obvious effects on P105/P50, p-IKKα/β and p-P65, and only significantly suppressed p-IκBα. DDP monotherapy

decreased p-IKKα/β, p-P65 and p-IκBα, but had no effect on P105/P50. Notably, combined treatment potently inhibited all four proteins, and its efficacy was significantly better than that of every single agent.

To determine whether ChR regulates EMT through the NF-κB pathway, PMA was employed to stimulate signaling pathways, including NF-κB. WB analysis in SKOV3/DDP cells showed that Twist1 expression was markedly reduced in the combination group, whereas PMA treatment significantly increased Twist1 expression (Fig. 7C and **Supplementary Fig. 2I**) and partially reversed the EMT-inhibitory effect of the combination therapy (Fig. 7D and **Supplementary Fig. 2J**). Moreover, PMA administration counteracted the suppressive effects induced by combined ChR and DDP treatment, as evidenced by reduced growth-inhibitory efficacy (Fig. 7E) and enhanced colony-forming capacity (Fig. 7F,G).

However, PMA is a broad-spectrum agonist that is not entirely specific to NF-κB; therefore, the above results provide supportive evidence for the involvement of the NF-κB pathway rather than demonstrating exclusive dependence. Overall, these findings indicate that ChR can help reverse cisplatin resistance in ovarian cancer cells, at least in part, by blocking EMT, which it achieves by suppressing the NF-κB/Twist1 signaling pathway.

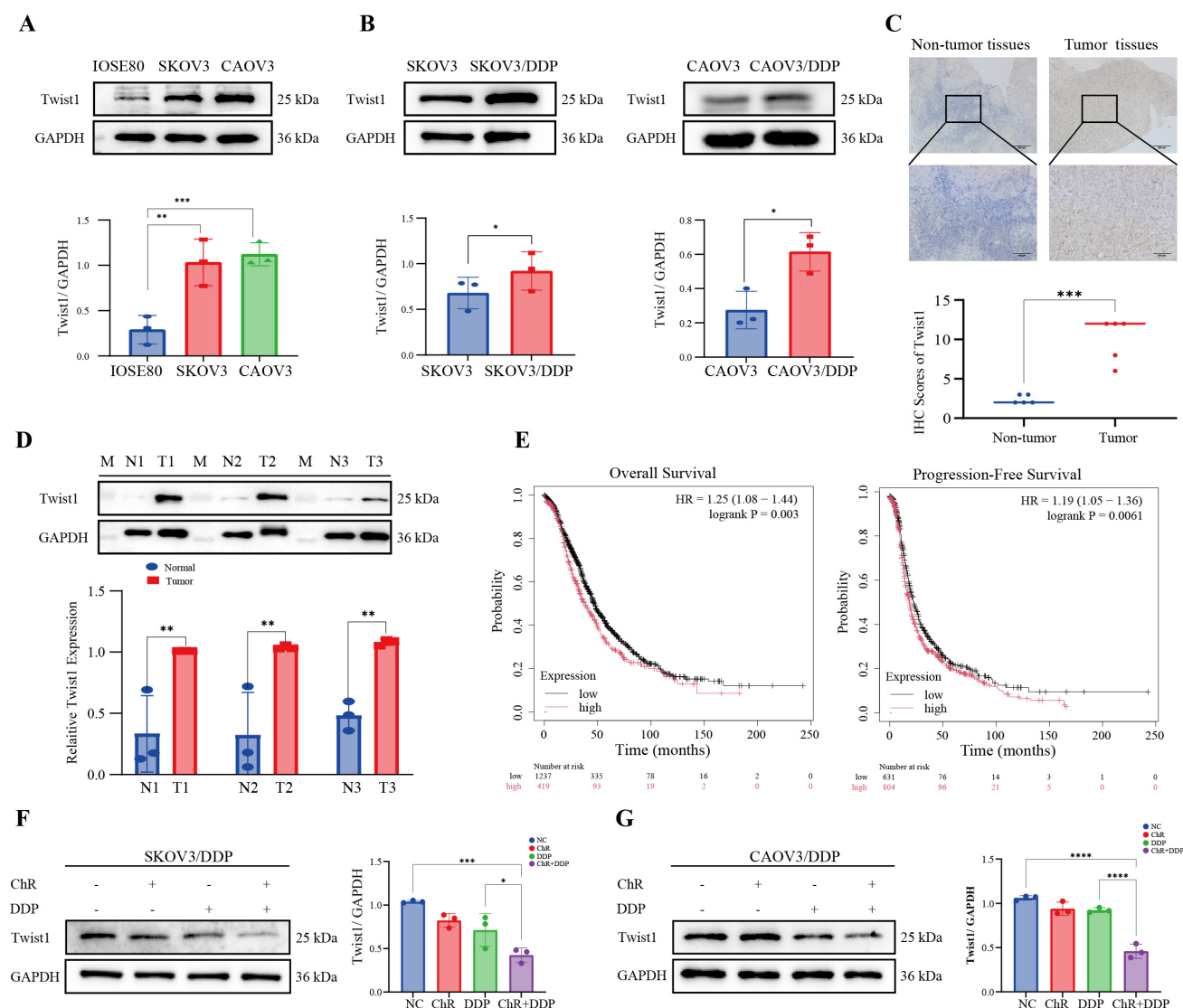


**Fig. 6. Twist1 is upregulated in cisplatin-resistant OC, correlates with poor prognosis, and responds to cisplatin treatment.** (A) Immunoblot detection of Twist1 in IOSE80 and OC cell lines. (B) Twist1 protein levels in parental versus cisplatin-resistant SKOV3/CAOV3 cells. (C) Representative IHC staining and H-score of Twist1 in OC specimens compared with non-malignant ovarian tissues. Scale bar: 200  $\mu$ m (upper), 100  $\mu$ m (lower). (D) WB analysis of Twist1 expression in OC tissues and non-tumorous ovarian tissues. “M” indicates the protein marker. (E) Kaplan-Meier survival curves demonstrated that high Twist1 expression was significantly correlated with overall survival (OS) and progression-free survival (PFS) in OC patients. (F) WB analysis of Twist1 expression in SKOV3/DDP cell under the indicated treatments. (G) WB analysis of Twist1 expression in CAOV3/DDP cells under the indicated treatments. Cell-based experiments were performed with  $n = 3$  independent replicates. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ . IHC, immunohistochemistry.

## 4. Discussion

Cisplatin resistance remains a major clinical problem in OC treatment. Here, we explored the ability of ChR to reverse cisplatin resistance in OC cells. Our findings indicate that ChR inhibits cell proliferation, induces apoptosis, and works synergistically with cisplatin at specific concentrations. These effects are associated with suppression of the NF- $\kappa$ B/Twist1/EMT pathway.

While ChR is known to possess antitumor effects, whether it can reverse DDP resistance in OC remains largely unknown. Here, we associate ChR with the NF- $\kappa$ B

pathway and Twist1, suggesting a potential mechanism underlying chrysin-induced chemosensitization. For example, shikonin was shown to synergize with cisplatin in ovarian cancer cells through ferroptosis induction [40]. Our findings demonstrate that this natural flavonoid exerts potent anti-ovarian cancer activity *in vitro* and enhances DDP efficacy, at least in part, by inhibiting the NF- $\kappa$ B pathway and suppressing EMT.

CompuSyn analysis showed that ChR (20  $\mu$ mol/L) and DDP (8  $\mu$ g/mL) worked synergistically. This concentration-dependent effect is consistent with the biphasic

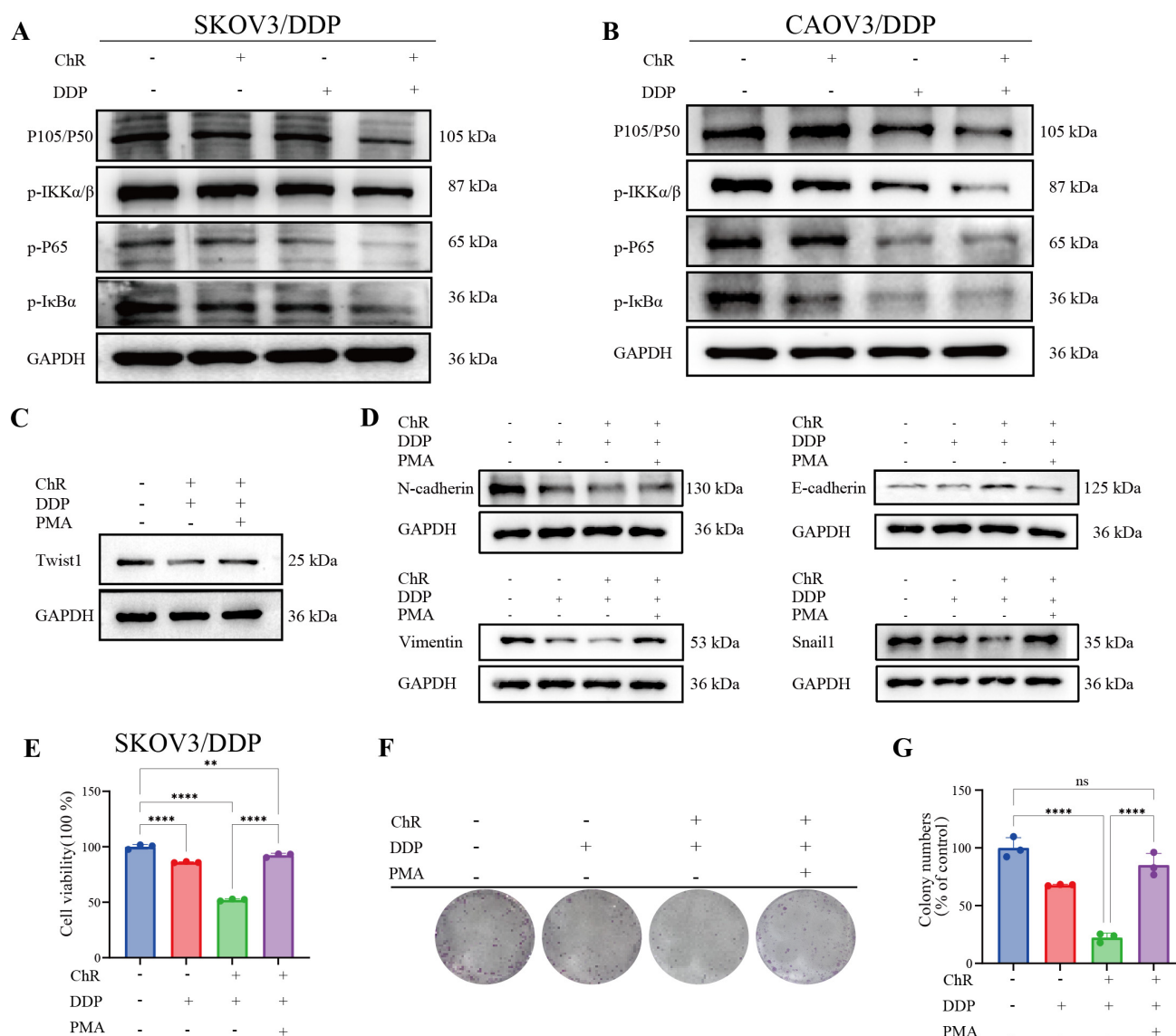


**Fig. 7. ChR reverses DDP resistance in OC via inhibition of the NF-κB signaling pathway.** (A) WB analysis of NF-κB pathway-related proteins in SKOV3/DDP cells under the indicated treatments. (B) WB analysis of NF-κB pathway-related proteins in CAOVS/DDP cells under the indicated treatments. (C) WB analysis of Twist1 expression in SKOV3/DDP cells under the indicated treatments. (D) WB analysis of EMT-related proteins in SKOV3/DDP cells under the indicated treatments. (E) Cell viability of SKOV3/DDP cells after drug treatment, measured by CCK-8 assay. (F) Colony formation assay evaluating the proliferative capacity of SKOV3/DDP cells.  $n = 3$ ,  $**p < 0.01$ ;  $****p < 0.0001$ ; ns, not significant. PMA, phorbol 12-myristate 13-acetate.

dose-response of flavonoids [41]. One possible explanation is that 20  $\mu\text{mol/L}$  ChR achieves an optimal balance between NF-κB inhibition and off-target effects, whereas higher doses may trigger competing stress responses that interfere with synergy. Regardless, careful dose optimization will be critical for any future application. We also recognize that ChR has poor bioavailability and that the effective concentrations used *in vitro* are relatively high. Therefore, we do not propose native ChR as a direct clinical candidate. Importantly, ChR showed minimal cytotoxicity toward normal ovarian epithelial IOSE80 cells at concentrations up to 200  $\mu\text{mol/L}$  (Supplementary Fig. 3), suggest-

ing a favorable safety profile. Nano-formulation or structural analogs may help improve its bioavailability and enable future translational studies.

EMT is a well-known biological process in which epithelial cells lose epithelial features and acquire mesenchymal properties, leading to increased migration and invasion. EMT activation promotes chemoresistance in many cancers [42,43,44,45,46], consistent with our observation that cisplatin-resistant OC cells show clear EMT reprogramming. Compared with parental cells, our resistant sublines exhibited upregulation of N-cadherin and Snail1, and downregulation of E-cadherin. Treatment with 20  $\mu\text{mol/L}$

ChR, especially in combination with DDP, reversed these changes, suggesting that ChR can inhibit EMT in resistant cells.

We also examined the NF- $\kappa$ B pathway, an upstream regulator of EMT. Reduced phosphorylation of IKK $\alpha$ / $\beta$ , I $\kappa$ B $\alpha$ , and p65, together with decreased P105/P50 and Twist1 expression, was detected in ChR-treated cells by Western blotting. To test whether NF- $\kappa$ B is functionally involved, we used PMA, a commonly used but non-specific activator of NF- $\kappa$ B. PMA partially counteracted the impact of ChR and DDP on cell survival and E-cadherin expression. Because PMA can activate multiple pathways, these results should be interpreted as supportive evidence rather than definitive proof. Nevertheless, the consistency between the transcriptomic data, Western blot validation, and partial PMA reversal suggests that the NF- $\kappa$ B/EMT axis is likely involved in ChR's effect.

Taken together, our findings suggest that ChR, particularly when formulated to improve bioavailability, holds promise for reversing chemoresistance in OC. Future studies should validate its *in vivo* efficacy using orthotopic xenograft models, improve bioavailability through nanoformulation, and investigate potential drug-drug interactions with cisplatin.

### Limitations

First, while the NF- $\kappa$ B/EMT cascade is known to mediate chemoresistance, the present study did not perform definitive genetic manipulations (e.g., knockdown or overexpression of key components) to establish causality. Therefore, our mechanistic conclusions remain largely correlative and should be interpreted with caution. Second, the working concentration of ChR *in vitro* is comparatively high, and its poor solubility and low bioavailability limit direct clinical translation. Third, the synergistic effect between ChR and cisplatin was observed only at specific concentration combinations; other ratios showed additive or weaker effects. The biological basis for this concentration-dependent synergy remains incompletely understood.

## 5. Conclusions

In summary, the present study demonstrates that ChR can reverse DDP resistance in OC cells. This effect is associated with suppression of the NF- $\kappa$ B/EMT pathway. Therefore, ChR's chemosensitizing action involves inhibition of this pathway. These results provide an initial *in vitro* basis for future studies aimed at improving ChR bioavailability and evaluating its chemosensitizing potential *in vivo*.

### Abbreviations

CAOV3/DDP, cisplatin-resistant CAOV3 cell line; ChR, chrysin; CI, combination index; DDP, cisplatin; EMT, epithelial-mesenchymal transition; Fa, Fraction of cells affected by combination therapy; IC<sub>50</sub>, half-maximal inhibitory concentration; IHC, Immunohistochemistry;

IOSE80, normal ovarian epithelial cells; MDR1, multidrug resistance protein 1; OC, ovarian cancer; PMA, phorbol 12-myristate 13-acetate; RI, resistance index; SKOV3/DDP, cisplatin-resistant SKOV3 cell line; WB, Western blot.

## Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author Contributions

NZ and XH were responsible for the conceptualization, methodology, investigation, formal analysis, data curation, and writing of the original draft. SL, XLu, YX, KJ, ML, JZ, and CZhu performed the investigation and collected the data. XLiu contributed to the formal analysis and writing—review & editing. CZhuang and TL contributed to the conception and design of the study and interpretation of the data, provided resources and academic guidance, and participated in the writing—review & editing. BC was responsible for the conceptualization, supervision, project administration, funding acquisition, and final writing—review & editing of the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University (Approval No: XYFY2025-KL271-01, Date: 06/25/2025). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study.

## Acknowledgment

We are sincerely grateful to the School of Medical Technology, Xuzhou Medical University, for providing the experimental platform, and to all teachers for their support during the experiments.

## Funding

This research was funded by the Medical Research Key Project of Jiangsu Provincial Health Commission (X20250914), the “Pairing Assistance” Research Project of the Affiliated Hospital of Xuzhou Medical University (FXJDBF2024220), the Xuzhou City Clinical Technical Backbone Training Program (2025211001), the Young Talent Program of The Affiliated Hospital of Xuzhou Medical University, the Advanced Program of The Affiliated Hospital of Xuzhou Medical University (PYJH2024210),

the Medical Research Project of Jiangsu Provincial Health Commission (H2023050), and the National Natural Science Foundation of China (No. 32301278).

## Conflicts of Interest

The authors declare no conflicts of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/CEOG52655>.

## References

- [1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a Cancer Journal for Clinicians*. 2021; 71: 209–249. <https://doi.org/10.3322/caac.21660>
- [2] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*. 2024; 74: 229–263. <https://doi.org/10.3322/caac.21834>
- [3] Caruso G, Weroha SJ, Cliby W. Ovarian Cancer: A Review. *JAMA*. 2025; 334: 1278–1291. <https://doi.org/10.1001/jama.2025.9495>
- [4] Gaillard S, Lacchetti C, Armstrong DK, Cliby WA, Edelson MI, Garcia AA, et al. Neoadjuvant Chemotherapy for Newly Diagnosed, Advanced Ovarian Cancer: ASCO Guideline Update. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2025; 43: 868–891. <https://doi.org/10.1200/JCO.24-02589>
- [5] Armstrong DK, Alvarez RD, Backes FJ, Bakkum-Gamez JN, Barroilhet L, Behbakht K, et al. NCCN Guidelines® Insights: Ovarian Cancer, Version 3.2022. *Journal of the National Comprehensive Cancer Network: JNCCN*. 2022; 20: 972–980. <https://doi.org/10.6004/jnccn.2022.0047>
- [6] St Laurent J, Liu JF. Treatment Approaches for Platinum-Resistant Ovarian Cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2024; 42: 127–133. <https://doi.org/10.1200/JCO.23.01771>
- [7] Karimzadeh MR, Masoudi Chelegahi A, Shahbazi S, Reisi S. Co-treatment of silymarin and cisplatin inhibited cell proliferation, induced apoptosis in ovarian cancer. *Molecular Biology Reports*. 2024; 51: 118. <https://doi.org/10.1007/s11033-023-09026-8>
- [8] Shirani A, Mirabbasi R, Shabani L, Reisi S. Pyrogallol acts as a novel anticancer factor to enhance the sensitivity to cisplatin in ovarian cancer cells through inducing miR-15a upregulation. *Egyptian Journal of Medical Human Genetics*. 2025; 26: 15. <https://doi.org/10.1186/s43042-025-00647-1>
- [9] Omidi F, Shahbazi S, Reisi S, Azhdari S, Karimzadeh MR. Glycyrrhizic acid enhances the anticancer activity of cisplatin in the human ovarian cancer cell line. *Toxicology in Vitro: an International Journal Published in Association with BIBRA*. 2023; 93: 105687. <https://doi.org/10.1016/j.tiv.2023.105687>
- [10] Jeong CY, Kim CE, Byun EB, Jeon J. Chrysin as a Bioactive Scaffold: Advances in Synthesis and Pharmacological Evaluation. *International Journal of Molecular Sciences*. 2025; 26: 9467. <https://doi.org/10.3390/ijms26199467>
- [11] Gong H, Song X, Yu J, Zhao X, Jiang H, Zhi X, et al. Chrysin: Phytochemistry, Pharmacology, Pharmacokinetics, Nano-Delivery Strategy, and Derivative Innovation. *The American Journal of Chinese Medicine*. 2025; 53: 2465–2488. <https://doi.org/10.1142/S0192415X25500909>
- [12] Zhang P, Yan B, Liu Y, Gu Y, Han M, Hu C, et al. Discovery of novel antiviral and antifungal agents based on flavonoid derivatives. *Bioorganic Chemistry*. 2025; 167: 109280. <https://doi.org/10.1016/j.bioorg.2025.109280>
- [13] Stompór-Gorący M, Bajek-Bil A, Machaczka M. Chrysin: Perspectives on Contemporary Status and Future Possibilities as Pro-Health Agent. *Nutrients*. 2021; 13: 2038. <https://doi.org/10.3390/nu13062038>
- [14] de Assis Carvalho ARB, Ferreira Damke GMZ, de Freitas Meirelles LE, Pantarotto Souza R, Ratti BA, Damke E, et al. Chrysin Exhibits Selective Antiproliferative and Antimigratory Activities in a Wide Range of Human-derived Cervical Cancer Cell Lines. *Anti-cancer Agents in Medicinal Chemistry*. 2025; 25: 1311–1322. <https://doi.org/10.2174/0118715206366318250312052617>
- [15] Xu Y, Tong Y, Ying J, Lei Z, Wan L, Zhu X, et al. Chrysin induces cell growth arrest, apoptosis, and ER stress and inhibits the activation of STAT3 through the generation of ROS in bladder cancer cells. *Oncology Letters*. 2018; 15: 9117–9125. <https://doi.org/10.3892/ol.2018.8522>
- [16] Han H, Lee SO, Xu Y, Kim JE, Lee HJ. SPHK/HIF-1 $\alpha$  Signaling Pathway Has a Critical Role in Chrysin-Induced Anticancer Activity in Hypoxia-Induced PC-3 Cells. *Cells*. 2022; 11: 2787. <https://doi.org/10.3390/cells11182787>
- [17] Chen Y, Zhao J, Wang S, Qi T, Hussain SA, Wu B. Apoptotic and anti-metastatic effect of chrysin on CD44<sup>+</sup> cancer stem cells from SW480 colorectal cancer cell line. *BioImpacts: BI*. 2025; 15: 31073. <https://doi.org/10.34172/bi.31073>
- [18] Chen Z, Li W, Jia B, Yin G, Sun Z, Tong Y, et al. Chrysin enhances sunitinib sensitivity in renal cell carcinoma by inducing ferroptosis via targeting PI3K/Akt/GPX4 pathway. *Toxicology and Applied Pharmacology*. 2025; 504: 117531. <https://doi.org/10.1016/j.taap.2025.117531>
- [19] Liu X, Zhang X, Shao Z, Zhong X, Ding X, Wu L, et al. Pyrotinib and chrysin synergistically potentiate autophagy in HER2-positive breast cancer. *Signal Transduction and Targeted Therapy*. 2023; 8: 463. <https://doi.org/10.1038/s41392-023-01689-w>
- [20] Karami-Dehkordi N, Reisi S, Feizi-Dehnavy M. Synergistic anticancer effects of tamoxifen in combination with fisetin and chrysin on breast cancer cells: insights into viability, migration, and apoptosis. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2026; 399: 10893–10908. <https://doi.org/10.1007/s00210-026-05076-4>
- [21] Wang Z, Li A, Song Z, Liu X, Ge Y, Chen Z, et al. CypA Mediates Non-Small Cell Lung Cancer Chemoresistance by Attenuating Ferroptosis via Stabilizing SLC7A11. *Advanced Science*. 2026; 13: e11947. <https://doi.org/10.1002/advs.202511947>
- [22] Chhipa AS, Gallicchio M, Boscaro V, Patel S. Synergistic anticancer effects of doxorubicin in combination with tilorone in breast cancer. *European Journal of Pharmacology*. 2025; 1008: 178249. <https://doi.org/10.1016/j.ejphar.2025.178249>
- [23] Lamture G, Crooks PA, Borrelli MJ. Actinomycin-D and dimethylamino-parthenolide synergism in treating human pancreatic cancer cells. *Drug Development Research*. 2018; 79: 287–294. <https://doi.org/10.1002/ddr.21441>
- [24] Jafari S, Ostadrahimi A, Farjami A, Montazersaheb S. Synergistic anticancer effects of Chrysin and trastuzumab in HER2-Positive breast cancer cells. *Scientific Reports*. 2025; 15: 41215. <https://doi.org/10.1038/s41598-025-25098-x>
- [25] Yan T, Zhang MM, Deng XY, Imam M, Ablise M, Zhang WY. Design and synthesis of novel quinoline-chalcone derivatives as dual inhibitors of tubulin polymerization and P-glycoprotein to overcome cisplatin resistance in cervical cancer. *Bioorganic*

- Chemistry. 2025; 165: 109006. <https://doi.org/10.1016/j.bioorg.2025.109006>
- [26] Wu L, Cai S, Deng Y, Zhang Z, Zhou X, Su Y, et al. PD-1/PD-L1 enhanced cisplatin resistance in gastric cancer through PI3K/AKT mediated P-gp expression. *International Immunopharmacology*. 2021; 94: 107443. <https://doi.org/10.1016/j.intimp.2021.107443>
- [27] Shi W, Wang F. circ\_AKT3 knockdown suppresses cisplatin resistance in gastric cancer. *Open Medicine (Warsaw, Poland)*. 2022; 17: 280–291. <https://doi.org/10.1515/med-2021-0355>
- [28] Xu L, Xu Y, Zheng J, Zhao Y, Wang H, Qi Y. Dacomitinib improves chemosensitivity of cisplatin-resistant human ovarian cancer cells. *Oncology Letters*. 2021; 22: 569. <https://doi.org/10.3892/ol.2021.12830>
- [29] Zhang CX, Huang RYJ, Sheng G, Thiery JP. Epithelial-mesenchymal transition. *Cell*. 2025; 188: 5436–5486. <https://doi.org/10.1016/j.cell.2025.08.033>
- [30] Han M, Zhou X, Cheng H, Qiu M, Qiao M, Geng X. Chitosan and hyaluronic acid in colorectal cancer therapy: A review on EMT regulation, metastasis, and overcoming drug resistance. *International Journal of Biological Macromolecules*. 2025; 289: 138800. <https://doi.org/10.1016/j.ijbiomac.2024.138800>
- [31] Jiang M, Wang J, Li Y, Zhang K, Wang T, Bo Z, et al. EMT and cancer stem cells: Drivers of therapy resistance and promising therapeutic targets. *Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy*. 2025; 83: 101276. <https://doi.org/10.1016/j.drug.2025.101276>
- [32] Sun Q, Xu J, Yuan F, Liu Y, Chen Q, Guo L, et al. RND1 inhibits epithelial-mesenchymal transition and temozolomide resistance of glioblastoma via AKT/GSK3- $\beta$  pathway. *Cancer Biology & Therapy*. 2024; 25: 2321770. <https://doi.org/10.1080/15384047.2024.2321770>
- [33] Zhang L, Zhao XL, Cao ZJ, Li KD, Xu LY, Tang F, et al. Ginsenoside CK inhibits EMT and overcomes oxaliplatin resistance in gastric cancer by targeting the PI3K/Akt pathway. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*. 2025; 140: 156516. <https://doi.org/10.1016/j.phymed.2025.156516>
- [34] Zhu W, Sun J, Jing F, Xing Y, Luan M, Feng Z, et al. GLI2 inhibits cisplatin sensitivity in gastric cancer through DEC1/ZEB1 mediated EMT. *Cell Death & Disease*. 2025; 16: 204. <https://doi.org/10.1038/s41419-025-07564-6>
- [35] Moghadam ER, Ang HL, Asnaf SE, Zabolian A, Saleki H, Yavari M, et al. Broad-Spectrum Preclinical Antitumor Activity of Chrysin: Current Trends and Future Perspectives. *Biomolecules*. 2020; 10: 1374. <https://doi.org/10.3390/biom10101374>
- [36] Roy S, Manna K, Jha T, Saha KD. Chrysin-loaded PLGA attenuates OVA-induced allergic asthma by modulating TLR/NF- $\kappa$ B/NLRP3 axis. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 2020; 30: 102292. <https://doi.org/10.1016/j.nano.2020.102292>
- [37] Li X, Huang Q, Ong CN, Yang XF, Shen HM. Chrysin sensitizes tumor necrosis factor- $\alpha$ -induced apoptosis in human tumor cells via suppression of nuclear factor- $\kappa$ B. *Cancer Letters*. 2010; 293: 109–116. <https://doi.org/10.1016/j.canlet.2010.01.002>
- [38] Yeo H, Lee YH, Koh D, Lim Y, Shin SY. Chrysin Inhibits NF- $\kappa$ B-Dependent *CCL5* Transcription by Targeting I $\kappa$ B Kinase in the Atopic Dermatitis-Like Inflammatory Microenvironment. *International Journal of Molecular Sciences*. 2020; 21: 7348. <https://doi.org/10.3390/ijms21197348>
- [39] Saleh DO, El-Nasr NMEA, Fayed AM, Ahmed KA, Mohamed RA. Uro-protective role of chrysin against cyclophosphamide-induced hemorrhagic cystitis in rats involving the turning-off NF- $\kappa$ B/P38-MAPK, NO/PARP-1 and STAT-3 signaling cascades. *Chemico-biological Interactions*. 2023; 382: 110585. <https://doi.org/10.1016/j.cbi.2023.110585>
- [40] Ni M, Zhou J, Zhu Z, Xu Q, Yin Z, Wang Y, et al. Shikonin and cisplatin synergistically overcome cisplatin resistance of ovarian cancer by inducing ferroptosis via upregulation of HMOX1 to promote Fe<sup>2+</sup> accumulation. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*. 2023; 112: 154701. <https://doi.org/10.1016/j.phymed.2023.154701>
- [41] Li Z, Wang H, Wang Y, Ma Z, Hu L, Luo S, et al. Luteolin inhibits the TGF- $\beta$  signaling pathway to overcome bortezomib resistance in multiple myeloma. *Cancer Letters*. 2023; 554: 216019. <https://doi.org/10.1016/j.canlet.2022.216019>
- [42] Tanaka N, Kosaka T, Miyazaki Y, Mikami S, Niwa N, Otsuka Y, et al. Acquired platinum resistance involves epithelial to mesenchymal transition through ubiquitin ligase FBXO32 dysregulation. *JCI Insight*. 2016; 1: e83654. <https://doi.org/10.1172/jci.insight.83654>
- [43] Erin N, Grahovac J, Brozovic A, Efferth T. Tumor microenvironment and epithelial mesenchymal transition as targets to overcome tumor multidrug resistance. *Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy*. 2020; 53: 100715. <https://doi.org/10.1016/j.drug.2020.100715>
- [44] Xu L, Zhou L, Wang Y, Jiang X, Cai Y. CCDC80 Induces Platinum Resistance in Ovarian Cancer Cells Through Epithelial-Mesenchymal Transition Pathway Activation. *Clinical and Experimental Obstetrics & Gynecology*. 2025; 52: 39988. <https://doi.org/10.31083/CEOG39988>
- [45] Grygilewicz P, Dymek B, Bujak A, Gunerka P, Stanczak A, Lamparska-Przybylska M, et al. Epithelial-mesenchymal transition confers resistance to selective FGFR inhibitors in SNU-16 gastric cancer cells. *Gastric Cancer: Official Journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association*. 2016; 19: 53–62. <https://doi.org/10.1007/s10120-014-0444-1>
- [46] Sousa-Squiavinato ACM, Arregui Ramos DA, Wagner MS, Tessmann JW, de-Freitas-Junior JCM, Morgado-Díaz JA. Long-term resistance to 5-fluorouracil promotes epithelial-mesenchymal transition, apoptosis evasion, autophagy, and reduced proliferation rate in colon cancer cells. *European Journal of Pharmacology*. 2022; 933: 175253. <https://doi.org/10.1016/j.ejphar.2022.175253>