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shRNA Depletion of cIAP1 Sensitizes Human Ovarian Cancer Cells to Anticancer Agent-Induced Apoptosis

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Emerging evidence suggests a potential role of cellular inhibitor of apoptosis protein 1 (cIAP1) in the development of human ovarian cancer. However, its function in the progression of ovarian cancer has not been clearly determined. Our study aimed to investigate the effect of cIAP1 gene depletion on the chemosensitivity of ovarian cancer cells. We developed a novel short hairpin RNA (shRNA) plasmid specifically targeting cIAP1. Cell proliferation, invasion, and apoptosis of the shRNA-transfected cells were evaluated using MTT, Transwell chamber, and flow cytometric assays, respectively. The concentration of MMP-9 in the supernatant was detected by ELISA. Targeted depletion of cIAP1 by shRNA significantly reduced expression levels of cIAP1 mRNA and protein, leading to inhibition of cell proliferation and invasion capability in SKOV3 cells. At the same time, cIAP1 downregulation decreased the secretion of MMP-9. shRNA depletion of cIAP1 enhanced chemosensitivity of ovarian cancer cells to Taxol and carboplatin-induced apoptosis. cIAP1 is associated with tumor progression in human ovarian cancer. Therefore, cIAP1 might be a potential target for therapeutic anti-cancer drugs.

Key words: Cellular inhibitor of apoptosis protein 1 (cIAP1); Ovarian cancer; RNA interference (RNAi); Cell proliferation; Invasion; Chemosensitivity; Taxol; Carboplatin

INTRODUCTION

Ovarian cancer is the third most common cancer of the female reproductive system, accounting for 2.4–5.6% of the morbidity of the common cancer in women worldwide, but its incidence rate is lower than cervical and endometrial cancers. However, the mortality of ovarian cancer is the highest one among the common cancers in women, due to the deficiency of effective early diagnostic methods for this disease (1). To cure ovarian cancer, the development of effective approaches for the investigation of morbidity factors, treatment regimens, and preservation measures is most urgently required. Therefore, a better understanding of molecular mechanisms underlying cell proliferation, invasion, and survival in ovarian cancer is critical for the development of therapeutic strategies. Emerging evidence suggests that cellular inhibitor of apoptosis protein 1 (cIAP1) is highly expressed in advanced stage ovarian cancer, implicating the potential role of cIAP1 in the progression of ovarian cancer (2).

The inhibitors of apoptosis (IAPs) are a family of proteins containing one or more characteristic baculovirus

IAP repeat (BIR) domains. These proteins have multiple biological functions, including activities to bind and inhibit caspases, regulate cell cycle progression, and modulate receptor-mediated signal transduction (3,4). It has been reported that there are eight members in the IAP family, which is a group of intracellular proteins containing one or more zinc-binding BIR domains. Among them, cIAP1 is the most important member, as it is a critical mediator of tumor necrosis factor- α (TNF- α)-induced nuclear factor (NF)- κ activation, and it is involved in the inhibition of death receptor-mediated apoptosis (5). The carboxyl-terminal RING domain of cIAP1 has ubiquitin E3 ligase properties. It has been reported that cIAP1 regulates receptor-mediated signaling pathways upstream of mitochondria through its distinct domain of BIR to interact with TNF receptor-associated factor 2 (TRAF2) (6). cIAP1 is also associated with caspase 3 and caspase 7 activities to regulate endogenous cell apoptosis (7). Since both cIAP1 and cIAP2 are target genes of NF- κ B (6,8), their expression levels are negatively correlated with TNF-induced cell death in NF- κ B-deficient cells. It has

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also been shown that activation of NF- κ B is required for the cIAP1- and cIAP2-mediated inhibition of TNF-induced cell death (9,10). It has been reported that TNF-stimulated MMP-9 gene expression is mediated through NF- κ B-mediated upregulating MMP-9 promoter activity (11). MMP-9 is known to play a crucial role in tumor invasion, angiogenesis, and subsequent distant metastasis by proteolytic degradation of ECM (12). According to those findings, NF- κ B can regulate the expression of IAPs, which may in turn regulate the NF- κ B signaling pathway, resulting in inhibition of apoptosis.

In recent reports, the sensitivity of tumor cells to radiotherapy and chemotherapy can be upregulated by downregulation of NF- κ B activity (13–15). Overexpression of cIAP1 in the advanced stage of ovarian cancer has been reported by Psyri et al. but lacks further reports related to the mechanism underlying the effect of cIAP1 in ovarian cancer (2). Therefore, clarifying the role of cIAP1 in human ovarian cancer is essential to the development of a novel therapeutic strategy for this disease.

The aim of the present study was to investigate a potential role for cIAP1 in regulating apoptosis in human ovarian cancer cells, particularly chemotherapeutic agent-induced apoptosis. We utilized RNA interference (RNAi) technology to downregulate cIAP1 gene expression in human ovarian cancer Skov3 cells. The gene depletion efficiency and changes in cellular proliferation, invasion, and apoptosis (also as a measure of chemosensitivity to Taxol and carboplatin) after cIAP1 knockdown in Skov3 cells were investigated. Our findings might provide experimental evidence in unfolding the molecular mechanisms underlying the progression of ovarian cancer as well as suggest a novel adjuvant therapy strategy to chemotherapy.

MATERIALS AND METHODS

Cell Culture and Reagents

Skov3, a human ovarian carcinoma cell line, was obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences (SIBS) of the Chinese Academy of Sciences (CAS). Cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Life Technologies, NY) supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO₂. Taxol was purchased from the Aosaikang Pharmaceutical Group (JiangSu, China). Carboplatin was purchased from Bristol-Myers Squibb S.p.A.

Development of cIAP1 shRNA and Its Transient Transfection

The vector pGPU6/GFP/Neo (Shanghai Sangon Biotech Co.) was used to establish an expression plasmid of shRNA that specifically targets cIAP1. The coding sequence of the

human cIAP1 gene was chosen from the Genebank database. Four sets of shRNA sequence-targeting human cIAP1 gene were listed as follows: (1) cIAP1-sh1839 (5'-GCTTGTTTCAGTGGTTCTTACT-3'); cIAP1-sh2098 (5'-GGATGATGCTATGTCAGAACA-3'); (2) cIAP1-sh2534 (5'-GGACCTGGAGAAAGTTCTTCA-3'); (3) cIAP1-sh2915 (5'-GCGAGAGAACTGATTGATACC-3'); (4) a nonspecific shRNA was designed as a negative control (cIAP1-shNC) (5'-GTTCTCCGAACGTGTCACGT-3'). The corresponding oligonucleotides were synthesized and then subcloned into the restriction sites of *Bam*HI and *S*I in the pGPU6/GFP/Neo vector. For transient transfection, Skov3 cells were plated in a six-well plate at a concentration of 6 × 10⁴ cells/well and incubated overnight. On the next day, cells were transfected with cIAP1 shRNAs using Lipofectamine™ 2000 (Invitrogen). Non-transfected Skov3 cells were also included as the blank control (mock) group.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from transfected and mock cells using the TRIzol® reagent (Invitrogen). The reverse transcription reaction was performed at 50°C for 15 min and finished by heating at 94°C for 5 min in a 20- μ l reaction system containing 3 μ g total RNA. The forward and reverse primers for cIAP1 were 5'-CCCAAAGACTTTTCC CAGGTCCCT-3' and 5'-ACTGAGCTTCCCACCACAGGCA-3', respectively. The sense and antisense primers for β -actin were 5'-CGTTGCTATC CAGGCTGTGCTA-3' and 5'-CCAGGTCCAGACGCA GGATGGC-3', respectively. The profile of RT-PCR is shown as follows: 30 cycles including denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 30 s. The final extension step was kept at 72°C for 10 min. The PCR products were subjected to electrophoresis on 1% agarose gels and visualized by ethidium bromide staining. Relative band intensities were detected using ImageJ software (NIH, Bethesda, MD).

Western Blot Analysis

Cells were lysed in chilled lysis buffer, followed by measurement of protein concentration in lysates of samples. Equal amounts of protein from each sample were separated by 10% SDS-PAGE and transferred onto PVDF membranes. The membrane was blocked by 5% fat-free milk in TBS-T at room temperature for 1 h. The blot was then incubated with anti-human cIAP1 mouse monoclonal antibody (1:400 dilution; Santa Cruz Biotechnology) overnight at 4°C, followed by 1-h incubation with horseradish peroxidase-conjugated anti-mouse goat monoclonal antibody (1:1,000 dilution; Santa Cruz Biotechnology) at room temperature. The protein bands were visualized using the DAB coloration kit and an

enhanced chemiluminescence (ECL) analysis system (Booster, China).

Assessment of Cell Proliferation

Cell proliferative potential was estimated using the methyl thiazolyl tetrazolium (MTT) assay. Exponentially growing cells were seeded into 96-well microtiter plates at a density of 5×10^4 cells per well. The next day, attached cells were transfected with the cIAP1 shRNA. At different time points, including 24, 48, 72, and 96 h after transfection, 20 μ l MTT (5 mg/ml; Sigma-Aldrich) dye was added to each well for an additional 4-h incubation at 37°C. The MTT reaction was stopped by aspiration of culture medium, and 150 μ l dimethyl sulfoxide (DMSO) (Sigma) was added to dissolve the formazan crystals. The spectrometric absorbance of each sample was measured at 490 nm using an automatic microplate reader (Bio-Rad Laboratories). MTT results were expressed as absorbance at 490 nm.

Matrigel Invasion Assay

Transwell chambers with 8- μ m pore size polycarbonate membranes (Costar) were coated with Matrigel (Becton-Dickinson) and incubated at 37°C for 30 min. For the invasion assay, 600 μ l RPMI-1640 with 10% fetal bovine serum (FBS) was added into the lower chamber, and 200 μ l cell suspension (5×10^4 cells) was added onto Matrigel in the upper chamber. Transwell chambers were cultured with serum-free RPMI-1640 for 24 h at 37°C. The Matrigel was scraped away, and the remaining polycarbonate membranes were stained with 0.1% crystal violet for 10 min. The number of invading cells passing through the Matrigel and attaching to the lower surface of the membranes was quantified using the MTT assay.

Enzyme-Linked Immunosorbent Assay (ELISA)

The concentration of MMP-9 in the culture supernatant was detected by enzyme linked immunosorbent assay (ELISA) kits (R&D, USA) according to the manufacturers' instructions. Cells were incubated for 48 h after transfection, and the culture supernatant was collected by centrifugation at 3,000 rpm for 15 min. Then standards and samples were pipetted into the coated wells, and MMP-9 present was bound by the immobilized antibody. The optical density was measured by ELISA reader at 450 nm, and the values were within the linear portion of the standard curve.

Assessment of Drug Sensitivity

The chemosensitivity of Skov3 cells to Taxol and carboplatin was evaluated using the MTT assay. Briefly, exponentially growing cells were seeded into 96-well plates at a plating density of 5×10^4 cells/well and incubated overnight. On day 2 posttransfection, the cells were treated with different concentrations of

Taxol or carboplatin for 24 h. Cell viability was measured by the MTT assay as described above. The spectrometric absorbance of each group was recorded to calculate the growth inhibition rate.

Flow Cytometric Analysis

Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining was used for apoptosis assessment in cells with cIAP1 knockdown and Taxol treatment or carboplatin treatment. Apoptotic cells were determined by flow cytometric analysis. At 48 h posttransfection, cells were treated with 10 μ g/ml Taxol or carboplatin for another 24 h. Cells were harvested, followed by staining with 5 μ l annexin V-FITC (20 μ g/ml) and 5 μ l PI (50 μ g/ml) (Sigma-Aldrich) for 15 min at 37°C. Detection of apoptotic cells was performed by flow cytometry (Beckman Coulter FC500).

Statistical Analysis

Statistical analyses were carried out using SPSS 20.0 software. Data are expressed as mean \pm standard deviation (SD). Statistical significance in each group was determined by Student–Newman–Keuls test. A value of $p < 0.05$ was considered to indicate statistically significant differences. Each experiment was repeated three times.

RESULTS

Transfection and Depletion Efficiency of cIAP1 shRNAs in Skov3 Cells

To evaluate transfection efficiency, Skov3 cells that were transfected with cIAP1 shRNAs were examined under a fluorescence microscope at 48 h after transfection. Results showed that in three randomly selected fields, more than 65% of cells displayed green fluorescence (Fig. 1A, B). Similarly, a 74.7% transfection rate was estimated by flow cytometric analysis (Fig. 1C, D). Depletion efficiency of cIAP1 shRNA in Skov3 cells was determined using RT-PCR and Western blot analyses. β -Actin was used as an internal control for both RT-PCR and Western blot analyses. Our results revealed that 48-h transfection of Skov3 cells with the version of sh2534 from cIAP1 shRNAs that we made achieved a significant knockdown of cIAP1 at the mRNA and protein levels, compared to the control cells. However, other versions of cIAP1 shRNAs had no obvious depletion effect in Skov3 cells (Fig. 2A, B). The comparison between sh2534 and the control is shown in Figure 2C–F: mRNA level, 0.48 ± 0.23 (sh2534) versus 0.84 ± 0.49 (control), and protein level, 0.41 ± 0.58 versus 1.25 ± 0.07 ($p < 0.01$). Because the effective depletion efficiency of cIAP1 shRNA (sh2534) had been confirmed in our preliminary experiments, we selected this shRNA construct as a tool to study the effect of shRNA knockdown of cIAP1 on the chemosensitivity of ovarian cancer cells.

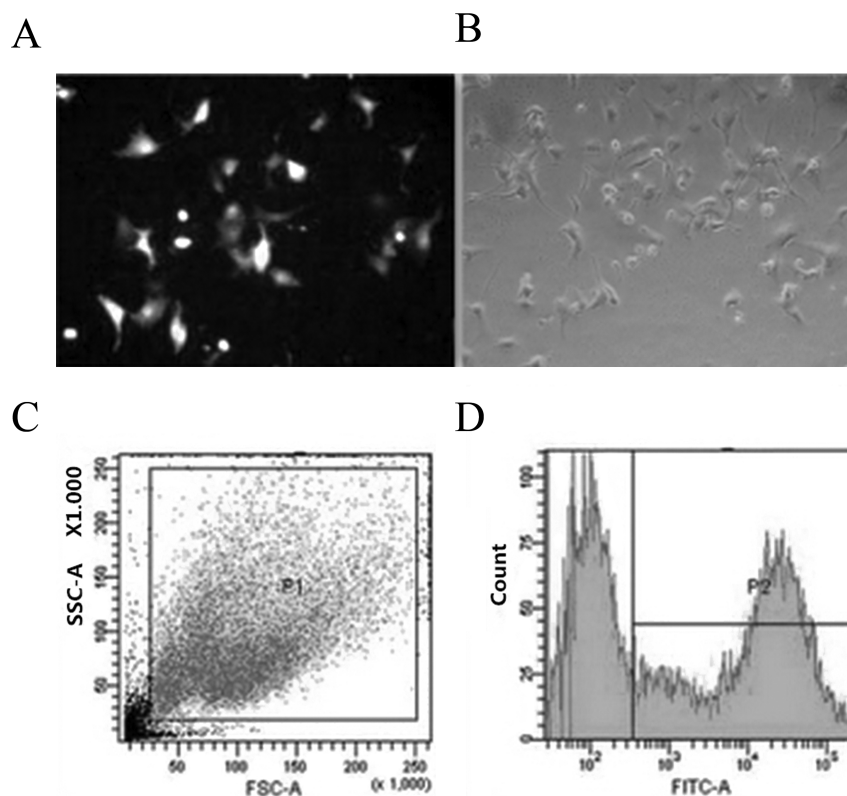


Figure 1. Evaluation of transfection efficiency of cIAP1 shRNAs in Skov3 cells. Representative images of Skov3 cells transfected with green fluorescent protein (GFP)-cIAP1 shRNA (sh2534) were captured by fluorescence (A) and phase-contrast (B) microscopy (magnification, 200 \times). The transfection efficiency of cIAP1 shRNAs in Skov3 cells was examined by flow cytometric analysis as described in the Materials and Methods (C and D).

Effect of cIAP1 Downregulation on Cell Proliferation and Cell Invasion Ability

As shown in Figure 3A, compared to the blank control group, cell viability in cIAP1 shRNA-transfected cells was greatly decreased. The growth inhibition rate was $44.47 \pm 0.052\%$ ($p < 0.05$), $48.70 \pm 0.075\%$ ($p < 0.05$), and $41.33 \pm 0.10\%$ ($p < 0.05$) at 48, 72, and 96-h posttransfection, respectively. No significant changes in cell viability of control cells at different time points were observed ($p > 0.05$), implying the downregulation of cell proliferation by shRNA depletion of cIAP1 in human ovarian cancer cells.

The data from the Matrigel invasion assay showed that the number of invading cells in the cIAP1 shRNA-transfected group was significantly less than that in the control group ($p < 0.01$), indicating that shRNA depletion of cIAP1 could affect the invasion ability of Skov3 cells. Meanwhile, there was no significant difference in the cellular invasion ability between the two control groups ($p > 0.05$) (Fig. 3B, C).

Furthermore, we detected the effect of cIAP1 downregulation on the production of MMP-9 by ELISA since MMP-9 plays a critical role in tumor cell invasion. As shown in Figure 3D, the concentration of MMP-9 in the

supernatant of the sh2534 group decreased extremely compared with the other two control groups 48 h after transfection ($p < 0.05$). No difference was seen between the blank control and shNC groups ($p > 0.05$). All these data suggest that cIAP1 plays an important role in modulating ovarian cancer cell invasion in part via its effect on MMP-9.

Knockdown of cIAP1 Enhances Tumor Chemosensitivity to Taxol and Carboplatin

To evaluate the effect of cIAP1 depletion on chemosensitivity of Skov3 cells to Taxol and carboplatin, survival rates of cells with Taxol treatment and carboplatin treatment in three groups including blank control, shNC, and cIAP1 shRNA were examined. As shown in Figures 4A and 5A compared to that in the blank control or shRNA groups, the cell viability in the cIAP1 shRNA group was significantly reduced at Taxol concentrations ranging between 1 and 20 $\mu\text{g/ml}$ ($p < 0.05$), as were the group cells of carboplatin treatment. Our results revealed that downregulation of cIAP1 expression through shRNA-mediated gene depletion might enhance the chemosensitivity of human ovarian cancer cells to Taxol treatment and carboplatin treatment.

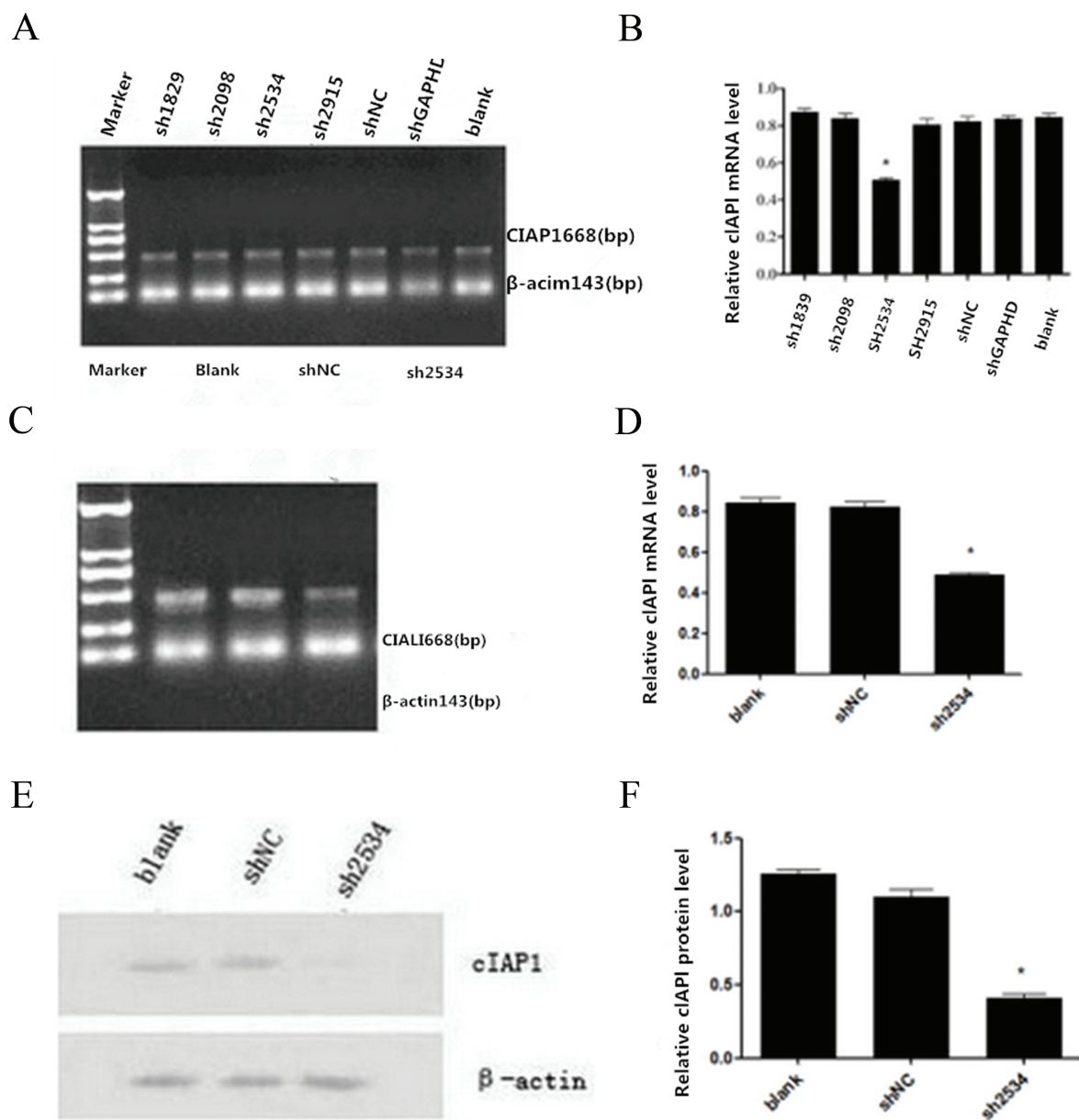


Figure 2. Evaluation of depletion efficiency of cIAP1 shRNA in Skov3 cells. (A) Using RT-PCR as described in the Materials and Methods, semiquantitative analysis of the relative mRNA levels of cIAP1 in Skov3 cells transfected with cIAP1 shRNAs was performed at 48-h posttransfection. (B) Comparison of the relative mRNA levels of cIAP1 between different versions of cIAP1 shRNA and controls including shNC, shGAPDH, and blank. (C and D) Representative semiquantitative analysis data of the relative cIAP1 mRNA level in sh2534-transfected cells, compared to shNC and blank control at 48-h posttransfection. (E and F) Representative Western blot data for cIAP1 expression in sh2534-transfected cells, compared to shNC and blank control at 48-h posttransfection. All data are expressed as mean ± SD. * $p < 0.05$ compared to blank control.

Increased Proportion of Apoptotic Cells Contributes to cIAP1 shRNA-Enhanced Chemosensitivity

To investigate the mechanisms underlying the cIAP1 shRNA-enhanced chemosensitivity, we examined the apoptotic cells in different treatment groups, using methods of annexin V-FITC/PI staining and flow cytometry. As shown in Figure 4B and C, treated with the same

concentration of Taxol (10 μg/ml), the percentages of apoptotic cells in three groups of blank control, shNC, and cIAP1 shRNA were 15.88%, 17.10%, and 24.08%, and as shown in Figure 5B and C, treated with the same concentration of carboplatin (10 μg/ml), the percentages of apoptotic cells in three groups of blank control, shNC, and cIAP1 shRNA were 15.35%, 16.95%, and 23.22%,

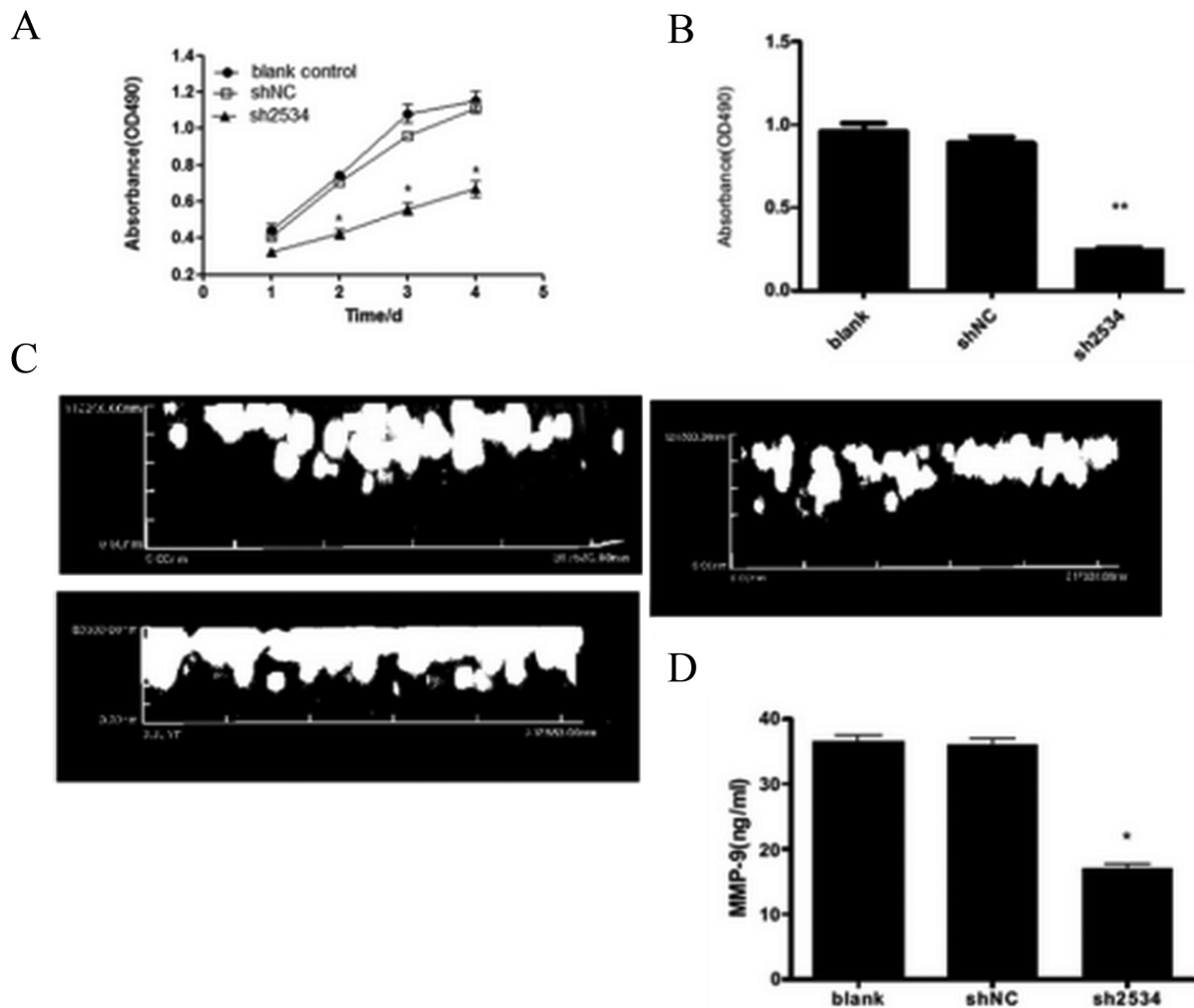


Figure 3. Effect of cIAP1 downregulation on cell proliferation and invasion ability. (A) The effect of cIAP1 downregulation on cell proliferation was determined by MTT assay as described in Materials and Methods. The cell growth curve was drawn according to the viability of cells with different transfections at the indicated time points. $*p < 0.05$ compared to blank control. Results of Matrigel invasion assay as described in the Materials and Methods. (B) Absorbance of invading cells measured by MTT assay and (C) representative images of the invading cells that penetrated the Matrigel to adhere to the membrane. $**p < 0.01$ compared to blank control or shNC. (D) cIAP1 knockdown inhibited the secretion of MMP-9. Representative ELISA of MMP-9 concentration in the supernatant posttransfection. $*p < 0.05$.

respectively, implying there were more apoptotic cells in the cIAP1 shRNA-transfected group than in the other two groups. The statistically significant difference in the number of apoptotic cells between cIAP1 shRNA and shNC or blank control was confirmed ($p < 0.01$), but the difference between the latter two groups was not significant ($p > 0.05$).

Taken together, our results indicate that shRNA depletion of cIAP1 could reduce the proliferative potential and invasion capability of human ovarian cancer cells. Chemosensitivity of ovarian cancer cells may be enhanced by cIAP1 knockdown through a mechanism associated with increased apoptosis. Our findings indicate

the potential role of cIAP1 in the development of human ovarian cancer and tumor chemosensitivity, with possible therapeutically important implications.

DISCUSSION

Optimal surgical debulking and postoperative chemotherapy are currently common techniques in the regimen for advanced ovarian cancer patients at stages III and IV. The current standard chemotherapeutic approach for ovarian cancer patients includes platinum-based regimens with or without taxanes. Although great progress in chemotherapy has been achieved, more than 50% of women with ovarian cancer still die of this disease. For

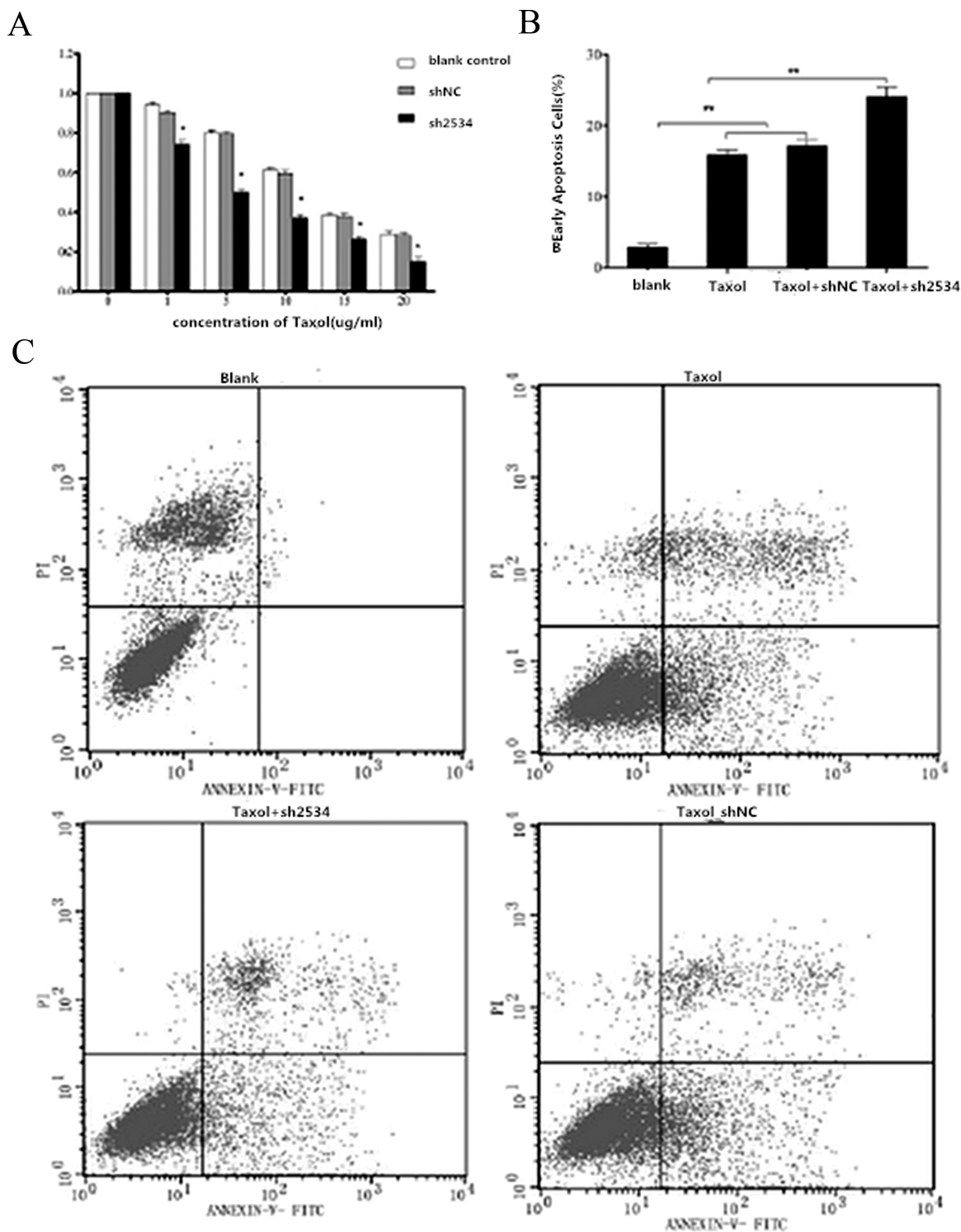


Figure 4. Knockdown of cIAP1 enhances chemosensitivity of Skov3 cells to Taxol treatment. (A) MTT assay results of the cIAP1 shRNA effect on the chemosensitivity of Skov3 cells to Taxol treatment. Cell viability is represented as a ratio of A490 (experimental group) versus A490 (negative control). * $p < 0.05$. (B) Quantitative analysis for the percentage of apoptotic cells, using annexin V, a cellular early apoptosis marker detected by flow cytometry. ** $p < 0.01$. (C) Representative flow cytometric data of apoptotic cells stained with annexin V-FITC/PI in the different groups.

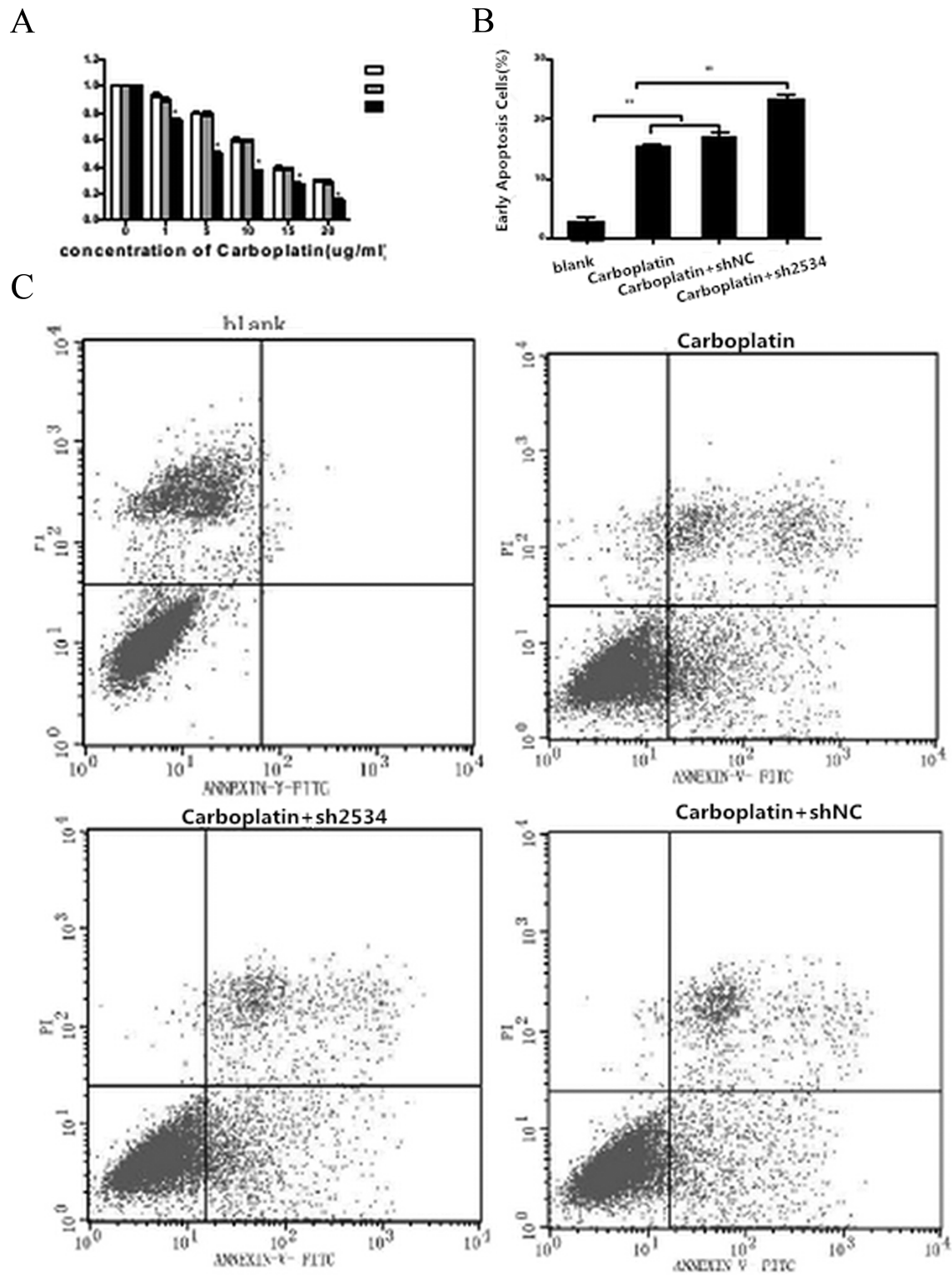


Figure 5. Knockdown of cIAP1 enhances chemosensitivity of Skov3 cells to carboplatin treatment. (A) MTT assay results of the cIAP1 shRNA effect on the chemosensitivity of Skov3 cells to carboplatin treatment. Cell viability is represented as a ratio of A490 (experimental group) versus A490 (negative control). * $p < 0.05$. (B) Quantitative analysis for the percentage of apoptotic cells, using annexin V, a cellular early apoptosis marker detected by flow cytometry. ** $p < 0.01$. (C) Representative flow cytometric data of apoptotic cells stained with annexin V-FITC/PI in the different groups.

this reason, identification of molecular targets for ovarian cancer treatment is extremely important, particularly in the era of molecular therapies.

Apoptosis occurring within ovarian cancer cells in response to chemotherapy is induced by the activation of effectors, resulting from the cleavage of the proteolytically inactive proforms of caspases 3, 6, and 7 (16,17). In the report by Tamm et al. (15), knockout of cIAP1 induced activation of caspase 3 and 7, leading to TRAIL signaling-mediated apoptosis and growth inhibition. This is because the proteolytic activity of caspase 3 is usually inhibited by cIAP1, which can directly bind to activated caspase 3 and 7 and attenuate their activities (18,19). As a result, the intrinsic pathway of apoptosis is subsequently initiated, resulting in a release of multiple death-promoting molecules from the mitochondria, such as holocytochrome c and Smac (20). It has been reported that cIAP1 contains three BIR domains, each consisting of approximately 70 amino acid residues. On the surface of the BIR domain, there are several hydrophobic regions with conserved charged amino acids that may be involved in the interaction of cIAP1 with other proteins. Mutational analysis indicated that the BIR domains are responsible for the inhibitory function of cIAP1 (21,22).

In the present study, we utilized RNAi technology to establish a novel cIAP1 shRNA expression plasmid. Human ovarian cancer Skov3 cells were transfected with this shRNA plasmid to deplete cIAP1. Our results confirmed the depletion efficacy of cIAP1 shRNA (sh2534) in Skov3 cells at both the mRNA and protein levels. MTT data showed that the proliferative potential of Skov3 cells transfected with cIAP1 shRNA was significantly decreased, compared to the control groups. Accordingly, the concentration of MMP-9 in the supernatant of cIAP1 sh2534 group decreased extremely. Our results implied that shRNA targeting cIAP1 could suppress the secretion of MMP-9; consequently, MMP-9 is one of the downstream target genes of cIAP1, which could promote invasion of ovarian cancer at least in part by regulating the expression of MMP-9. MMP-9 plays an important role in tumor invasion via proteolytic degradation of major components of basement membrane. A previous study reported that members of the IAP family function through binding to and inhibition of several caspases (23,24). Activation of the intrinsic/mitochondrial apoptosis pathway leads to disruption of MMP-9 and release of apoptogenic proteins, such as cytochrome c, which removes cIAP1 blockage of caspase activation (25,26).

Psyri et al. reported that the 3-year survival rate of ovarian cancer patients with high expression of cIAP1 is lower than that of patients with lower expression of cIAP1 (2). In another report, a low expression level of membranous cIAP1 was the only significant prognostic factor for

overall survival in multivariable analysis, adjusting for well-characterized prognostic variables (27). Imoto et al. reported that among nine cell lines, two cell lines have cIAP1 amplification and overexpression. Compared to the cell lines without cIAP1 amplification, these two cell lines are highly resistant to radiation-induced cell death. In an immunohistochemical (IHC) study with specimens from 70 patients with primary cutaneous squamous cell carcinomas (CSCCs) who only received radiotherapy, both overall survival and local recurrence-free survival rates in the patients with tumors that had a high expression level of nuclear cIAP1 were significantly lower than those in patients whose tumors had less or no nuclear expression of cIAP1. Multivariate analysis also implicated that cIAP1 may be an important factor that contributes to the development/progression of this disease. Therefore, cIAP1 could be used as a novel biomarker to individually predict possible radiotherapy resistance in CSCC patients (28). Our study found that depletion of cIAP1 by shRNA could induce growth inhibition in Skov3 cells, which is highly consistent with previous reports that showed the expression level of cIAP1 correlated with the proliferative potential of tumor cells.

Historically, Taxol and carboplatin are the most active drugs for ovarian cancer treatment. It is generally believed that Taxol and carboplatin exhibit their cytotoxic properties by preferentially binding to nuclear DNA to disturb normal transcription processes, and/or DNA replication mechanisms, leading to induction of apoptosis. Therefore, increased repair activity of platinum-DNA adducts might be a real cause of Taxol resistance and carboplatin resistance. Thus, eliminating the DNA repair activity in Taxol-treated and carboplatin-treated cells might enhance the apoptosis. Among the antiapoptotic molecules affecting cancer cell survival, the product of the cIAP1 gene is thought to be an important contributor to carcinogenesis. Correlation of cIAP1 overexpression with resistance to chemotherapy-induced apoptosis has been found in different tumor cell lines derived from a variety of cancer tissues (29,30). Our data also showed that knockdown of cIAP1 could enhance the chemosensitivity of ovarian cancer cells to Taxol and carboplatin. Flow cytometric analysis revealed that Taxol-induced and carboplatin-induced apoptosis could be increased in the cIAP1-depleted cells. Our results are consistent with those of other reports. Although direct evidence for the role of cIAP1 in tumorigenesis is currently lacking, our results support the hypothesis that cIAP1 as a potent regulator of cell death may play important roles in carcinogenesis. Further studies to clarify the mechanisms underlying our findings as well as in vivo experiments to evaluate the role of cIAP1 in ovarian cancer development are warranted.

We conclude that our homemade cIAP1-targeting shRNA can significantly reduce cIAP1 expression at the

mRNA and protein levels, leading to an inhibition of proliferative potential and invasion capability in ovarian cancer cells. Furthermore, cIAP1 knockdown also enhanced the chemosensitivity of ovarian cancer cells by increasing apoptosis. Our findings suggest that cIAP1 is a potential target for chemotherapeutic drugs and provides new insight into a gene therapy strategy for ovarian cancer treatment.

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