NDRG2 gene expression pattern in ovarian cancer and its specific roles in inhibiting cancer cell proliferation and suppressing cancer cell apoptosis

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Research

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Abstract

Background The cancer cell metastasis and the acquisition of chemotherapy resistance remain huge challenge for ovarian cancer treatment. Previously, N-myc downstream-regulated gene 2 (NDRG2) serves as a tumor suppressor for many cancers. Here, we attempted to investigate the specific roles of NDRG2 in ovarian cancer.

Methods The expression levels of NDRG2 were detected by qRT-PCR or Immunoblotting assay. CCK-8 assay was employed to examine the cell viability of ovarian cancer cells. The colony formation ability was determined by colony formation assay. Flow cytometry analyses were performed to detect the cell apoptosis and cell cycle.

Results Herein, we revealed that NDRG2 mRNA expression and protein levels were downregulated within both ovarian cancer tissues and cell lines. The overexpression of NDRG2 dramatically inhibited the cell viability and colony formation, whereas promoted the cell apoptosis and cell cycle arrest in G1 phase within ovarian cancer cells. More importantly, NDRG2 overexpression significantly enhanced the suppressive roles of cisplatin (DDP) in ovarian cancer cell viability. On the contrary, NDRG2 silence exerted opposing effects on ovarian cancer cells.

Conclusions In summary, we provide a solid experimental basis demonstrating the tumor-suppressive effects of NDRG2 in inhibiting the cell proliferation, enhancing the cell apoptosis, eliciting the cell cycle arrest in G1 phase, and promoting the suppressive effects of DDP on the viability of ovarian cancer cells. NDRG2 administration presents a potent adjuvant treatment for ovarian cancer therapy, which needs further in vivo and clinical investigation.

Introduction

Ovarian cancer is one of the deadliest malignancies in female [1, 2]. Since the incipient symptoms of ovarian cancer are obscure, most patients received diagnosis until entering the advanced stage [3–5]. Surgical treatment and platinum-based chemotherapy are major therapeutic strategies for ovarian cancers [6]. Unfortunately, these therapeutic methods seem to become less effective with the progression of the cancer. Moreover, the morbidity of the ovarian cancer also remains a higher level due to lack of reliable predictive biomarker, ovarian cancer cells metastasis and the resistance to chemotherapy [7]. Thus, it’s necessary to determine ovarian cancer pathophysiology and find new treatment methods.

Interestingly, it has been revealed the correlations between cancer (such as lung, prostate, liver, colorectal and breast cancer) and N-myc downstream-regulated gene 2 (NDRG2) [8–10]. NDRG2 is considered to be a tumor suppressor which contributes to not only hormone, ion, fluid metabolism and other cellular metabolic processes [11, 12], but also stress responses, like those under hypoxic environments and lipid toxicity [13, 14]. It has been demonstrated the correlations between NDRG2 and cancer within neurotumors [15, 16], gastroenteric tumors [9, 17], genitourinary tumors [18, 19], breast carcinoma [20, 21], lung carcinoma [10, 22], thyroid carcinoma [23], oral squamous-cell cancer [24], myeloid leukemia [25],

and cervical cancer [14]. Collectively, the expression of NDRG2 is reduced within human tumors, while its overexpression suppresses the capacity of cancer cells to proliferate, migrate, metabolize and invade [26]; NDRG2 expression levels are negatively correlated with human cancer clinical and pathological conditions [26]. Nevertheless, little is known about the specific role of NDRG2 within ovarian cancer.

Herein, NDRG2 mRNA and protein expression showed to be monitored within ovarian cancer tissues and cells. Next, NDRG2 overexpression and silence were conducted in three cell lines of ovarian cancer; the specific effects of NDRG2 upon the viability, colony formation ability, apoptosis, cell cycle, and the sensitivity to cisplatin (DDP) treatment of ovarian cancer cells were evaluated. In summary, we attempt to provide a solid experimental basis for understanding the cellular functions of NDRG2 on ovarian cancer cells.

Materials And Methods

Clinical tissue samples

We collected a total of 6 paired non-cancerous (NC) and ovarian cancer tissues from patients received resection surgeries in Zhongshan hospital with the signed the consent from each patient. All the experiments in the present study were conducted with the approval of the Ethics Committee of Zhongshan hospital. The pathologic type of all samples was confirmed by two independent pathological experts. Tissues were frozen at -80°C immediately after sampling until further use.

Cell lines and cell culture

A normal cell line, human ovarian surface epithelial cell line (HOSE, also known as HOSEpiC), was purchased from ScienCell (Cat. #7310; Carlsbad, CA, USA) and cultured in Ovarian Epithelial Cell Medium (OEpiCM, Cat. #7311; ScienCell). Ovarian cancer SKOV3 (ATCC® HTB-77™), OVCAR-3 (ATCC® HTB-161™), and CAOV3 (ATCC® HTB-75™) cell lines were obtained from ATCC (Manassas, VA, USA). SKOV3 cells were cultured in McCoy's 5a Medium Modified (Catalog No. 30-2007; ATCC). CAOV3 cells were cultured in Dulbecco's Modified Eagle's Medium (Catalog No. 30-2002; ATCC). OVCAR-3 cells were cultured in RPMI-1640 Medium (Catalog No. 30-2001; ATCC). All the cells were cultured with 10% FBS (Invitrogen, Carlsbad, CA, USA) at 37℃ in 5% CO_2.

Cells were transfected with scramble siRNA (negative control, si-NC; RiboBio, Guangzhou, China) or NDRG2 siRNA (si-NDRG2; RiboBio) with the help of Lipofectamine 3000 reagent (Thermo Fisher Scientific, Waltham, MA, USA). Cells were collected and used for further experiments 48 h after transfection.

PCR-based analyses

Total RNA was extracted from target cells with the help of TRIzol reagent (Invitrogen). The reverse transcription of extracted RNAs into cDNA was performed with the help of Maxima First Strand cDNA

Synthesis Kits (K1672; Thermo Fisher). The expression of mRNA was determined with an SYBR® Green Real-time PCR Master Mix (Sigma, St. Louis, MO, USA) using GAPDH as an endogenous control. All the results were processed and analyzed using the $2^{-\Delta\Delta Ct}$ method.

Immunoblotting

Cell lysate was prepared using RIPA lysis buffer (Applygen, Beijing, China) and proteins were extracted. SDS-PAGE (10%) was used to separate the extracted proteins. After that, proteins were transferred onto PVDF membranes. Nonspecific antigen was blocked by 5% non-fat milk solution, and the membranes were washed three times using PBST. The membranes were then incubated overnight at 4°C with anti-NDRG2 (12015-1-AP, Proteintech, Rosemont, IL, USA) and anti-β-actin (60008-1-Ig, Proteintech). After washing with PBST, the membranes were incubated with appropriate HRP goat anti-mouse/anti-rabbit IgG (Proteintech) at room temperature. The visualization of all the blots were conducted using enhanced chemiluminescence (ECL; Thermo Fisher).

Cell viability determined by CCK-8 assay

A CCK-8 kit (Sigma-Aldrich) was employed to examine the cell viability of ovarian cancer cell lines in response to different treatment and/or transfection. Cells were placed into 96-well plates at a density of 1×10^4 cells/well. CCK-8 solution was added at 0 h and 24 h thereafter and then cells were incubated for 4 h at 37°C. The absorbance (OD value) was measured at 450 nm.

Colony formation

The colony formation ability was determined. Cells were cultured 6-well plates at a density of 1×10^2 cells/well. Fourteen days later, the colonies were fixed with methanol and stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). After that, the number of visible colonies were counted.

Cell cycle and cell apoptosis determined by Flow cytometry

Flow cytometry analyses were performed to detect the cell apoptosis and cell cycle. For cell apoptosis analysis, cells were collected, resuspended, and added with annexin V-FITC and PI. After 15 min of incubation, the cell apoptosis was analyzed.

For cell cycle analysis, cells were fixed with ethanol (70%, ice-cold) for 20 min and added with PI. After 20 min incubation, the cell cycle was analyzed.

Data processing and statistical analysis

All data collected from three independent experiments were processed and analyzed by GraphPad (San Diego, CA, USA). The data was represented as mean ± SD. The comparison was conducted using paired or unpaired Student's *t*-test. A *P* value of less than 0.05 was considered as statistically significant.

Results

The mRNA and protein expression of NDRG2 within tissues and cells

To further confirm how NDRG2 affected ovarian cancer, we first verified NDRG2 expression within the tissues and cells of ovarian cancer. The mRNA expression of NDRG2 showed to be dramatically downregulated within ovarian cancer tissues than that in non-cancerous tissue samples (Fig.1A); similarly, the mRNA expression of NDRG2 also showed to be remarkably downregulated within three ovarian cancer cells, SKOV3, OVCAR-3, and CAOV3, than that in a normal cell line, HOSE (Fig.1C). Consistently, NDRG2 protein levels were decreased within ovarian cancer tissues than those in non-cancerous tissue samples (Fig.1B), and decreased in SKOV3, OVCAR-3, and CAOV3 cells, compared to those in HOSE cells (Fig.1D).

Involvements of NDRG2 in proliferation, apoptosis, and cell cycle of ovarian cancer cells

To further investigate the specific effects of NDRG2 on ovarian cancer cells, we conducted NDRG2 overexpression and NDRG2 silence in SKOV3, OVCAR-3, and CAOV3 cells by transfection with vector (negative control), NDRG2 OE, si-NC (negative control), or si-NDRG3. The transfection efficiency was determined via real-time PCR (Fig.2A-B).

Next, the effects of NDRG2 overexpression and silence on ovarian cancer cells were evaluated. As revealed by CCK-8 and colony formation analyses, NDRG2 overexpression significantly suppressed, whereas NDRG2 silence promoted the cell viability and colony formation ability of SKOV3, OVCAR-3, and CAOV3 cells (Fig.3A-B). Consistently, NDRG2 overexpression significantly enhanced, whereas NDRG2 silence suppressed cell apoptosis within all the three ovarian cancer cells (Fig.4A). Moreover, NDRG2 overexpression significantly induced the cell cycle arrested in G1 phase, whereas NDRG2 silence exerted an opposing effect (Fig.4B). These data indicate that NDRG2 inhibits the cell viability and colony formation, and induces apoptosis and cell cycle arrest in G1 phase, thus acting as a tumor suppressor within ovarian cancer cells.

Effects of NDRG2 on ovarian cancer cell sensitivity to DDP treatment

To date, DDP is one of the most valid agents of ovarian cancer and other solid tumors [27-29]. After confirming the tumor-suppressive effect of NDRG2 on ovarian cancer cells, we further investigated whether NDRG2 could sensitize ovarian cancer cells to DDP treatment. We transfected SKOV3, OVCAR-3, and CAOV3 cells with vector (negative control), NDRG2 OE, si-NC (negative control), or si-NDRG2 under DDP treatment and examined for cell viability. As shown in Fig.5, NDRG2 overexpression enhanced the suppressive role of DDP in the viability of ovarian cancer cells, whereas NDRG2 silence exerted an opposing effect. In summary, NDRG2 could improve the cellular effects of DDP on ovarian cancer cells.

Discussion

Herein, we revealed that NDRG2 mRNA expression and protein levels were downregulated within both ovarian cancer tissues and cell lines. The overexpression of NDRG2 dramatically inhibited the cell viability and colony formation, whereas promoted the cell apoptosis and cell cycle arrest in G1 phase within ovarian cancer cells. More importantly, NDRG2 overexpression significantly enhanced the suppressive roles of DDP in ovarian cancer cell viability. On the contrary, NDRG2 silence exerted opposing effects on ovarian cancer cells.

NDRG2 is one member of the NDRG family that contains NDRG1, NDRG2, NDRG3, and NDRG4 [30, 31]. NDRG2 has been reported to be reduced within colorectal cancer, liver cancer, thyroid cancer, glioblastoma, breast cancer and a number of other human cancers [16, 17, 20, 23, 32, 33]. Moreover, the increase in NDRG2 expression within cancer cells can be related to improved prognosis in gastric cancer, high-grade glioma and hepatocellular carcinomas [17, 34, 35]. Herein, the mRNA and protein expression of NDRG2 showed to be dramatically downregulated within the ovarian cancer tissues than that in the normal controls. In vitro, the mRNA and protein expression of NDRG2 also showed to be remarkably downregulated within SKOV3, OVCAR-3, and CAOV3 cells than those within a non-cancerous ovarian epithelial cell line, HOSE. Since excessive NDRG2 protein within cancer cells leads to significantly decreased cell proliferation [16, 36, 37] while the increased mRNA expression of NDRG2 can be related to improved prognosis [17, 34], we speculate that NDRG2 might act as a tumor suppressor within ovarian cancer, possibly by regulating ovarian cancer cell proliferation and apoptosis.

To verify the above-mentioned speculation, we conducted NDRG2 overexpression or silence in ovarian cancer cells and evaluated its cellar effects. Consistent with its expression pattern, NDRG2 acts as a tumor suppressor within ovarian cancer cell lines. NDRG2 overexpression dramatically suppressed the proliferation whereas enhanced the apoptosis of ovarian cancer cells, similarly to its effects on other cancers [22, 38–40]. Besides, the resetting of the G1, S, G2, M phases and other cell cycles can be found in the occurrence and development of tumors. As confirmed by a GO (Gene Ontology) enrichment analysis on the biological process, molecular function and cellular component, the overexpression of NDRG2 can increase the G protein signaling-associated genes while reduce the M phase-associated gene sets, which is consistent with cell cycle analyses [41]. Analyses on the signaling pathways have also revealed the decreased glycosylphosphatidylinositol (GPI)-anchor biosynthesis and protein degradation [41]. It has also been demonstrated by Ma et al. [19] that G1 arrest can be induced by the expression of NDRG2. NDRG2 was introduced into SW620 cells, after which the cell cycle arrest was observed to arrest at G1/S phase [37]. The expression of NDRG2 can effectively inhibit cell cycle resetting within tumors. Herein, we observed similar results that, NDRG2 overexpression dramatically induced the cell cycle arrest in G1 phase in all the three ovarian cancer cell lines, suggesting that NDRG2 might suppress ovarian cancer cell proliferation via affecting the resetting of cancer cell cycle.

Cisplatin is the most common platinum-based chemotherapy drug. Its action mechanism is DNA cross-linking, so its tumor-suppressive activity is broad-spectrum and non-cell cycle-specific, leading to the inhibition of DNA replication and transcription and induction of tumor cell apoptosis [42, 43]. Although cisplatin has been considered an effective agent for ovarian cancer treatment, the acquisition and

development of drug resistance has emerged as a primary obstacle to its wide clinical application [44]. Previously, Liu et al. [45] reported that NDRG2 silence inhibits the expression of Bcl-2, so that cervical cancer Hela cells can be sensitive to cisplatin. In histiocytic lymphoma U937 cells, NDRG2 could modulate NOX5-ROS-PKR pathway-regulated Bak-to-Mcl-1 ratio to increase the sensitivity to cisplatin [46]. In the present study, NDRG2 overexpression within ovarian cancer cell lines significantly enhanced the suppressive effects of DDP upon the viability of ovarian cancer cells, whereas NDRG2 silence exerted an opposing effect. These data indicate that NDRG2 might sensitize ovarian cancer cells to DDP treatment, which needs further in vivo experimental investigation.

Conclusion

Taken together, our findings provide a solid experimental basis demonstrating the cellular effects of NDRG2 in inhibiting the cell proliferation, enhancing the cell apoptosis, eliciting the cell cycle arrest in G1 phase, and promoting the suppressive effects of DDP upon the viability of cancer cells. Nevertheless, it is still necessary to further study the new strategy of rescuing the abnormally downregulated NDRG2 expression as a promising therapeutic strategy for clinical applications.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Zhongshan hospital. All participants signed the informed consent.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Conflict of Interest

The authors declare that they have no competing interest.

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Not applicable.

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Figures

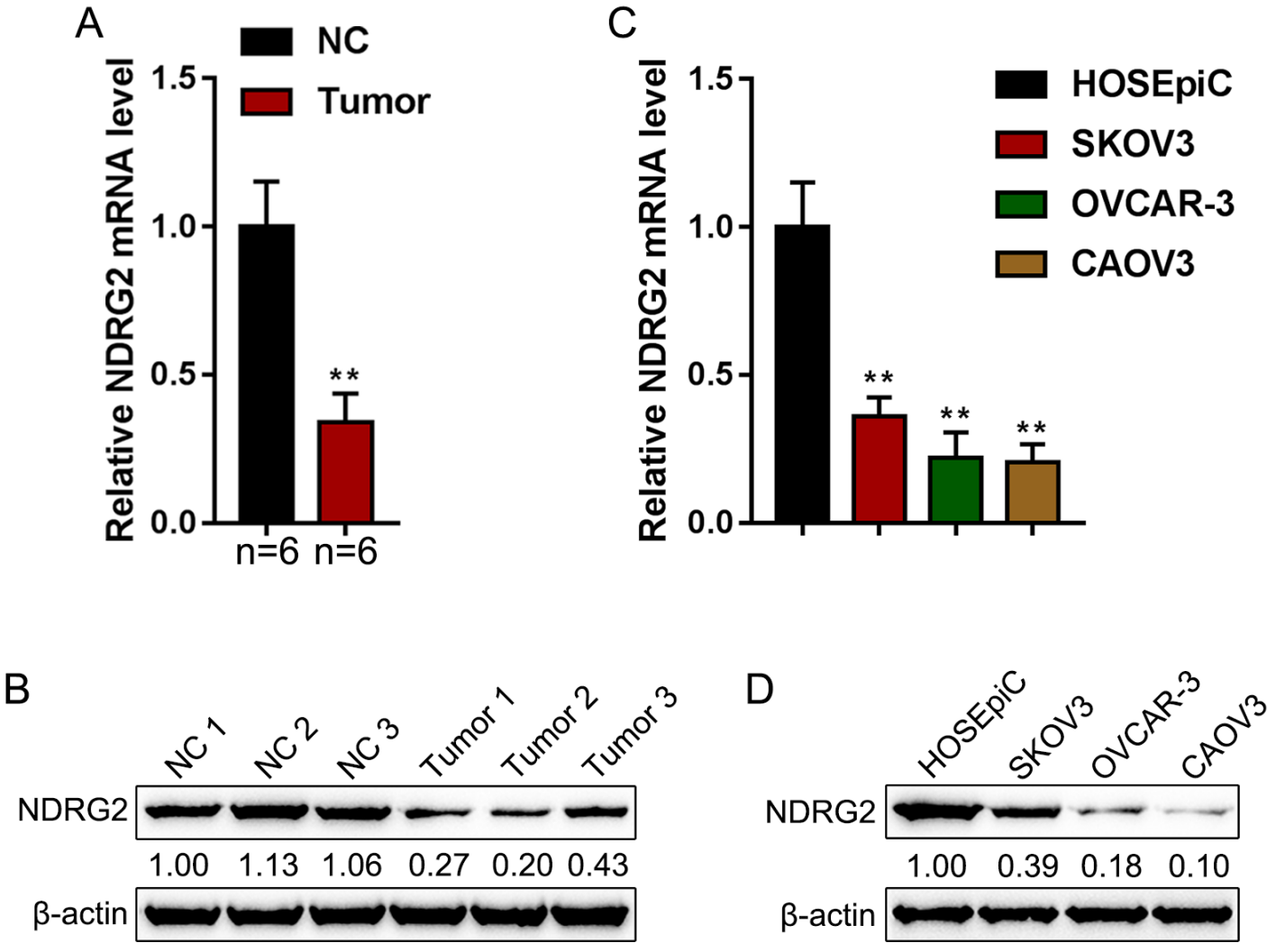

Figure 1

NDRG2 mRNA expression and protein levels in tissue samples and cell lines (A and C) NDRG2 mRNA expression was determined in 6 paired non-cancerous and tumor tissues, and in one normal cell line and three ovarian cancer cell lines, SKOV3, OVCAR-3, and CAOV3 by real-time PCR. (B and D) NDRG2 protein levels were determined in 6 paired non-cancerous and tumor tissues, and in one normal cell line and four ovarian cancer cell lines by Immunoblotting. **P<0.01.

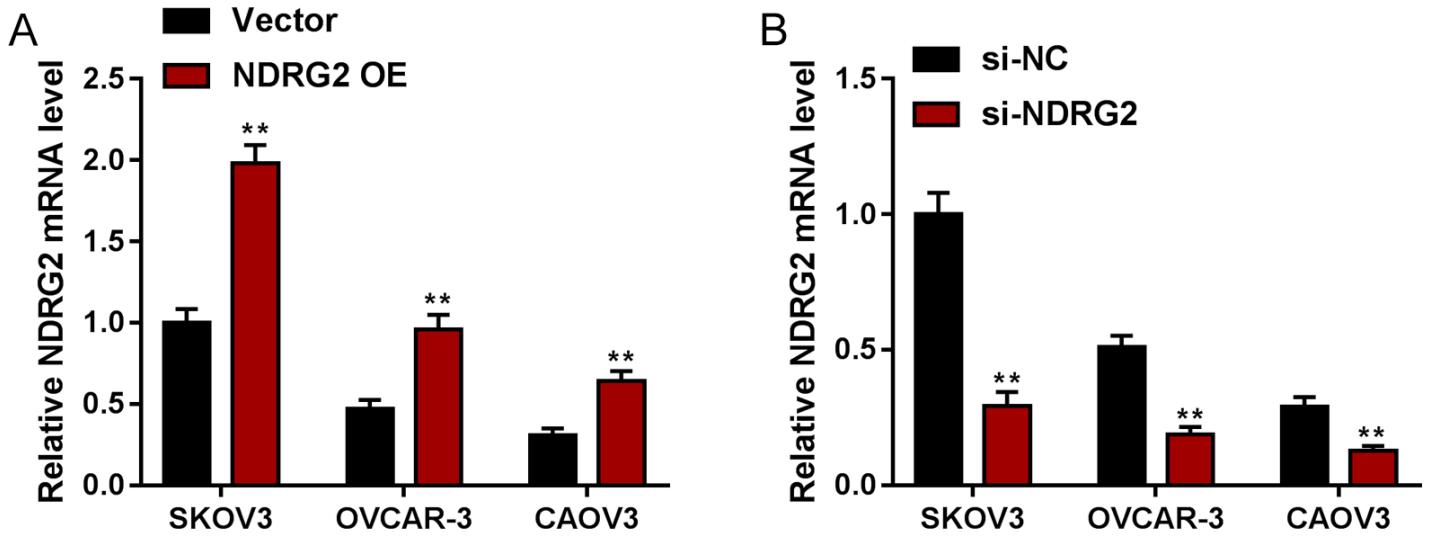

Figure 2

NDRG2 overexpression or silence in ovarian cancer cells (A) SKOV3, OVCAR-3, and CAOV3 cells were transfected with vector (negative control) or NDRG2 OE, as confirmed by real-time PCR. (B) SKOV3, OVCAR-3, and CAOV3 cells were transfected with si-NC (negative control) or si-NDRG2, as confirmed by real-time PCR. **P<0.01.

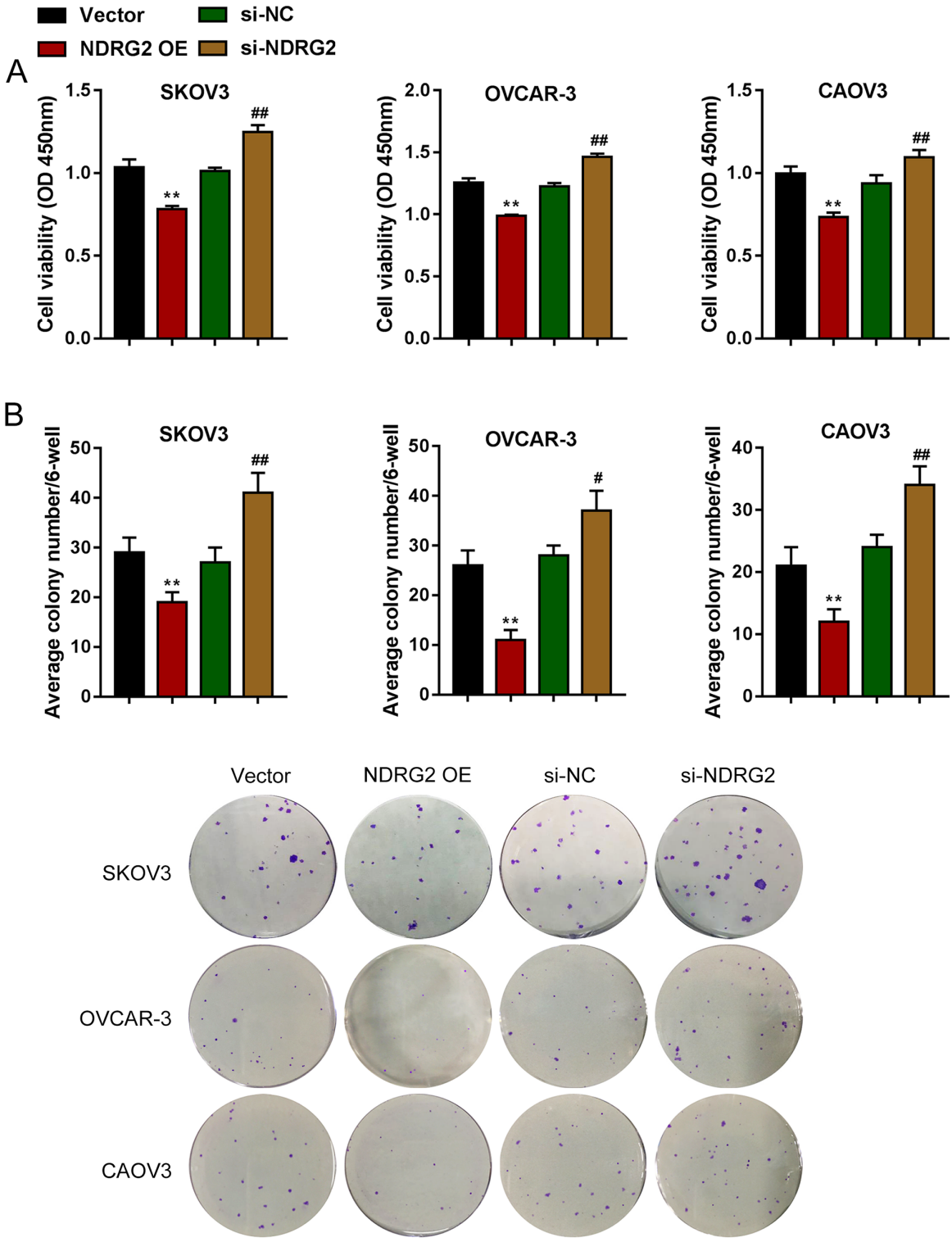

Figure 3

Effects of NDRG2 on ovarian cancer cell proliferation SKOV3, OVCAR-3, and CAOV3 cells were transfected with vector (negative control), NDRG2 OE, si-NC (negative control), or si-NDRG3 and examined for (A) cell viability by CCK-8; (B) colony formation ability. **P<0.01, compared to Vector group; ##P<0.01, compared to si-NC group.

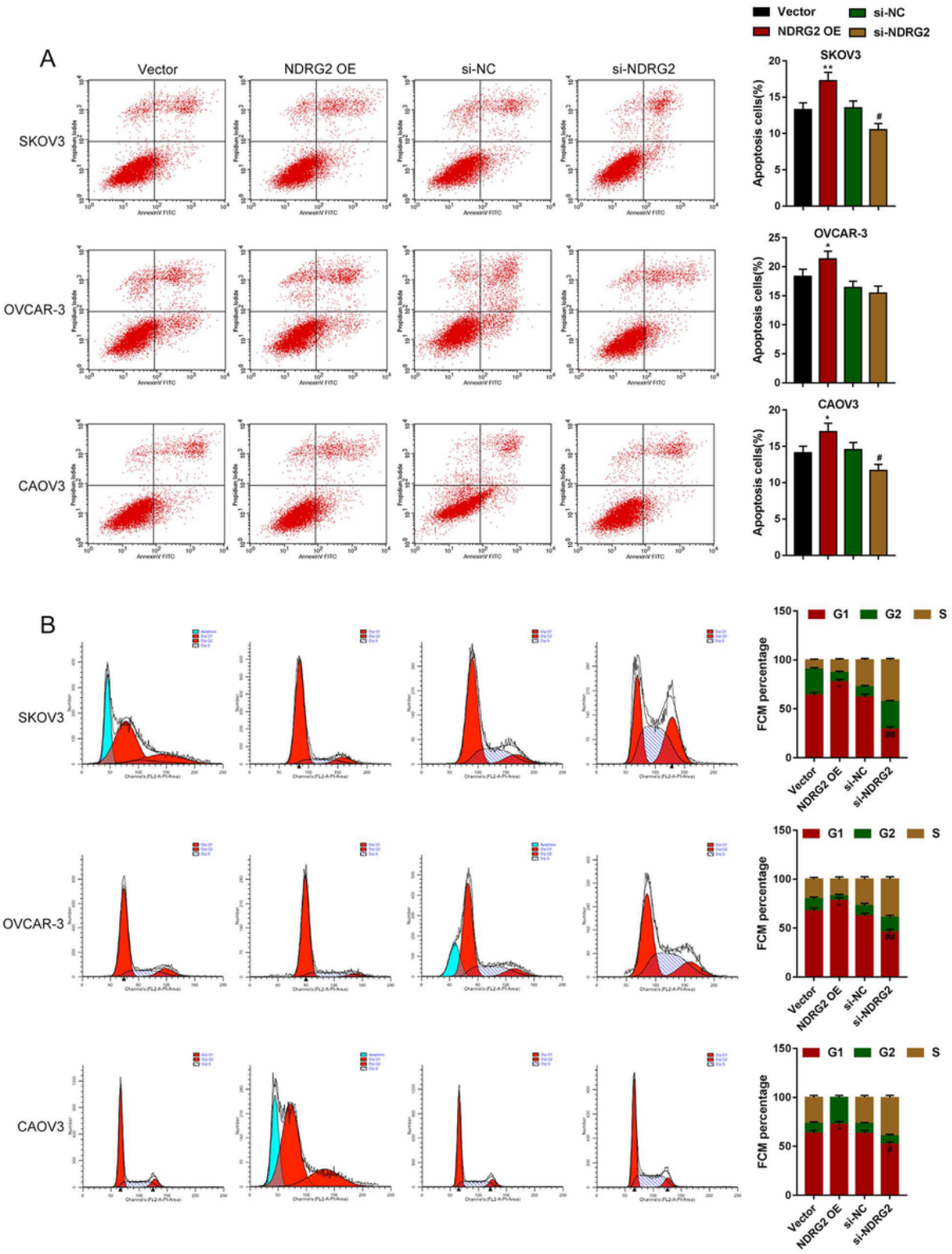

Figure 4

Effects of NDRG2 on ovarian cancer cell apoptosis and cell cycle SKOV3, OVCAR-3, and CAOV3 cells were transfected with vector (negative control), NDRG2 OE, si-NC (negative control), or si-NDRG3 and examined for (A) cell apoptosis and (B) cell cycle by Flow cytometry. **P<0.01, compared to Vector group; ##P<0.01, compared to si-NC group.

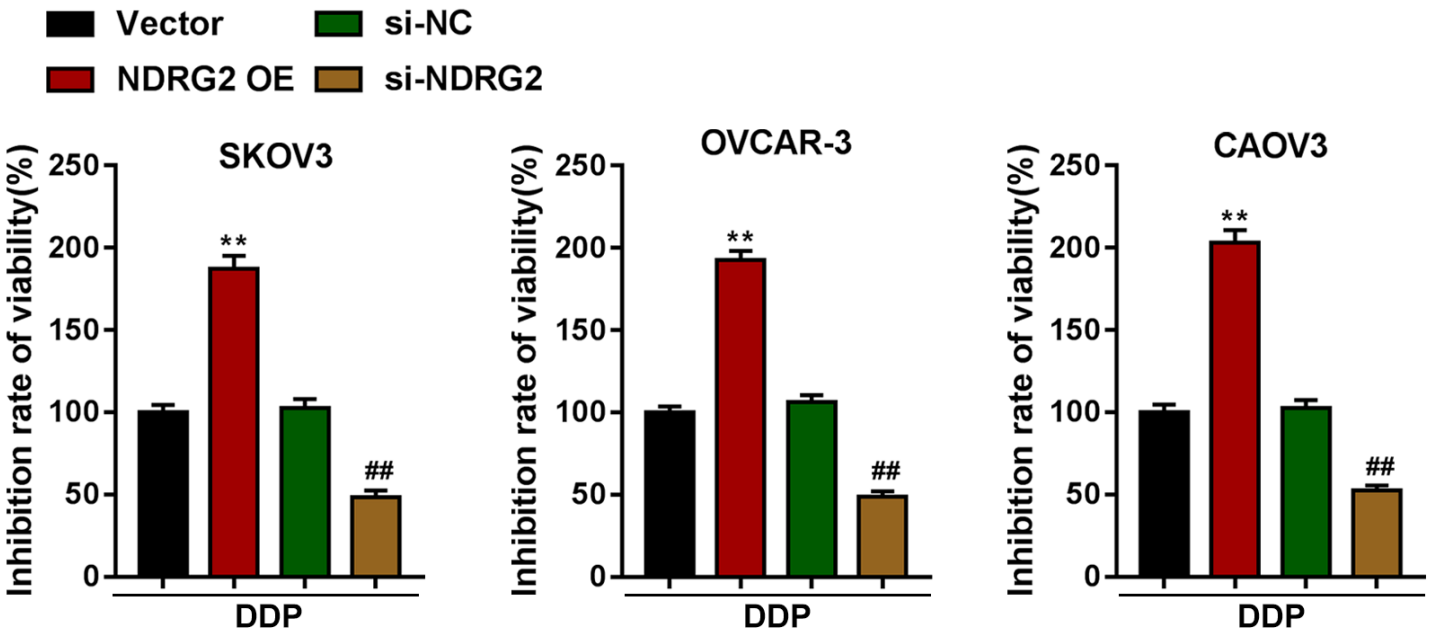

Figure 5

Effects of NDRG2 on ovarian cancer cell sensitivity to DDP treatment SKOV3, OVCAR-3, and CAOV3 cells were transfected with vector (negative control), NDRG2 OE, si-NC (negative control), or si-NDRG3 under DDP treatment and examined for cell viability by MTT analysis. **P<0.01, compared to Vector group; ##P<0.01, compared to si-NC group.