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RESEARCH ARTICLE

BSV163/DOPE-mediated TRAIL gene transfection acts synergistically with chemotherapy against cisplatin-resistant ovarian cancer

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Abstract

Ovarian cancer is the seventh most frequently diagnosed cancer among women worldwide. Most patients experience recurrence and succumb eventually to resistant disease, underscoring the need for an alternative treatment option. In the presented manuscript, we investigated the effect of the TRAIL-gene, transfected by an innovative bioinspired lipid vector BSV163/DOPE in the presence or absence of cisplatin, to fight against sensitive and resistant ovarian cancer. We showed that BSV163/DOPE can transfect ovarian cancer cell lines (Caov3, OVCAR3, and our new cisplatin-resistant, CR-Caov3) safely and efficiently. In addition, TRAIL-gene transfection in association with cisplatin inhibited cellular growth more efficiently (nearly 50% in Caov3 cells after the combined treatment, and 15% or 25% by each treatment alone, respectively) owing to an increase in apoptosis rate, caspases activity and TRAIL's death receptors expression. Most importantly, such synergistic effect was also observed in CR-Caov3 cells demonstrated by an apoptosis rate of 35% following the combined treatment in comparison with 17% after TRAIL-gene transfection or 6% after cisplatin exposition. These results suggest this combination may have potential application for sensitive as well as refractory ovarian cancer patients.

Abbreviations: CR, charge ratio; DISC, death-inducing signaling complex; DR, death receptor; FADD, Fas-associated death domain protein; RLU, relative light unit; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

Frédérique d'Arbonneau and Tristan Montier contributed equally to this study.

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KEYWORDS

BSV163/DOPE, cisplatin-resistance, lipid vector, ovarian cancer, TRAIL, transfection

1 | INTRODUCTION

Ovarian cancer is the third most frequent gynecologic cancer worldwide with the highest mortality rate predicted to increase significantly by 2040 (Coburn et al., 2017). Ovarian cancer symptoms are nonspecific. About 70% of patients are detected at an advanced stage when the 5-year relative survival rate is only 29% (Brett et al., 2017; Foley et al., 2013). Following first-line treatments including mainly optimal cytoreductive surgery and platinum/taxane-based systemic therapy, a clinical remission occurs in the majority of patients. Unfortunately, most of them will develop recurrence and ultimately fall victim to chemo-resistant disease, especially cancers resistant to platinum salts which are largely considered the backbone of ovarian cancer management (Foley et al., 2013). To improve anti-cancer efficacy and overcome drug resistance, research has shifted nowadays toward targeted biological therapy. Two targeted therapies have been approved by FDA and EMA, namely, poly-ADP-ribose polymerase inhibitors and anti-vascular endothelial growth factor monoclonal antibodies (Guan & Lu, 2018). In recent years, cancer gene therapy has also attracted great attention owing to the continued progress made in nucleic acids delivery techniques (Nguyen et al., 2023). However, the application of gene therapy as monotherapy in the treatment of ovarian cancer has not been as successful as had been expected (Áyen et al., 2018).

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) belongs to the TNF cytokine superfamily. Its extracellular domain can be cleaved by proteases releasing a soluble and functional form (Alizadeh Zeinabad & Szegezdi, 2022). TRAIL induces cell death upon ligation with its death receptors (Singh et al., 2021). The discovery of its potential to explicitly target malignant cells while sparing normal cells has been met with great enthusiasm, even though the underlying mechanisms have not yet been thoroughly understood (Nguyen et al., 2023). It has been reported that most primary and residual ovarian tumors expressed at least one TRAIL death receptor (DR4 and DR5), while in residual tumors following chemotherapy, one of the two death receptors of TRAIL—DR5 was more frequently observed (Arts et al., 2004; Dong et al., 2008; Duiker et al., 2010). While promising, the use of recombinant forms of TRAIL as well as agonistic antibodies of its receptors has not achieved the expected outcomes in

clinical trials (von Karstedt et al., 2017). Thereafter, alternatives such as TRAIL gene therapy have been examined to exploit the potential of this antitumor agent (Zhong et al., 2019).

Recently, we have reported the synthesis and characterization of an innovative nano-based bioinspired cationic lipid vector BSV163/DOPE (Bouraoui et al., 2020; Ghanem et al., 2023). BSV163/DOPE has been used successfully to transfect different cell lines and has demonstrated effectiveness and safety in in vivo experiments (Ghanem et al., 2023). In this study, using BSV163/DOPE, we aimed to investigate the killing effect of BSV163/DOPE-based TRAIL gene transfection in association with cisplatin treatment in different ovarian cancer cell lines as well as its cisplatin-resistant analogs.

2 | MATERIALS AND METHODS

2.1 | Cell lines and culture

Caov3 (ATCC HTB-75) and OVCAR3 (ATCC HTB-161) human ovarian cancer cell lines were purchased from American Type Culture Collection. These cell lines were chosen based on their accessibility and genetic similarity with high-grade serous ovarian cancer—the most frequent subtype of ovarian cancer (Domcke et al., 2013). Cisplatin-resistant Caov3 (CR-Caov3) cells were obtained by exposing Caov3 cells to increasing concentrations of cisplatin over 6 months (from 1.7 to 2.5 μM). Cells were cultured in RPMI medium supplemented with 10% FBS, 1% L-glutamine and 1% penicillin–streptomycin and incubated at 37°C and 5% CO₂.

2.2 | Plasmids

TRAIL-encoding plasmid (pVAX2-TRAIL, 3771 bp) was kindly provided by INSERM UMR 1238. Firefly luciferase-encoding plasmid (pGM144, 3759 bp) used as a transfection control plasmid was obtained from the University of Oxford. The plasmids were amplified in *Escherichia coli* M61665 and extracted with NucleoBond PC 10,000 purification kit (Macherey-Nagel). The purity of plasmids was verified by electrophoresis on 0.8% agarose gel. In every instance, plasmids were used only if it was predominantly supercoiled. DNA concentrations were determined by measuring the absorption at

260 nm. All plasmids used had an A260/A280 ratio between 1.8 and 2.

2.3 | Preparation of BSV163-based liposomal formulations

The ramified lipid vector BSV163/DOPE is synthesized by a film dispersion method as previously reported (Bouraoui et al., 2020; Ghanem et al., 2023). Briefly, BSV163 and DOPE (1:1, molar ratio) were dissolved in chloroform. The solution was evaporated using rotary evaporator at low temperature until the solvent was removed. Afterward, the formed lipid film was hydrated in sterile water at 4°C overnight. The lipid suspension was next sonicated to obtain a homogenized solution. Liposomal solutions were finally prepared at the concentration of 1.5 mM.

2.4 | Plasmid complexation and relaxation assay

The pDNA complexation and relaxation capability of BSV163/DOPE were evaluated by agarose gel electrophoresis. pVAX2-TRAIL was encapsulated by the vector in water at different charge ratios (CRs 2–5). After 30 min of complexation at room temperature, dextran sulfate (20 mg/mL) was added and incubated with the mixture for an additional 60 min to allow pDNA relaxation. Each sample was then deposited in a 0.8% agarose gel stained with ethidium bromide nuclei acids gel (Dutscher). A visualization by UV transilluminator (Fisher Bioblock) was performed following 20 min of migration at 100 V.

2.5 | Nanoparticles characterization

Zetasizer Nano ZS (Malvern Panalytical) was used to determine the hydrodynamic diameter, polydispersity index, and zeta potential of the lipid vector BSV163/DOPE as well as its related complexes with pVAX2-TRAIL at CRs 2–5. All samples were diluted with distilled water (1:10) before being measured at room temperature. The values calculated were mean of three measurements and mean values \pm SD were reported.

2.6 | In vitro gene transfection

Cells were transfected using BSV163/DOPE-plasmids complexes as demonstrated elsewhere (Bouraoui et al., 2019; Le Corre et al., 2014). In short, cells were seeded in 96-wells or 24-wells culture plates and were

transfected upon reaching 80% confluency by adding lipoplexes, formed by spontaneous self-assembly of BSV163/DOPE and 0.25 or 1 μ g DNA at different CRs 2–5, to each well. The commercial lipid vector Lipofectamine 3000 was used as a control for transfection efficiency. Following transfection with luciferase-encoding pGM144, the in vitro transfection properties (including efficiency and cytotoxicity) were determined as described previously (Khalil et al., 2021; Le Guen et al., 2021). Briefly, in each well, luciferase activity measured by the luciferase assay system kit (Promega) was normalized by the protein content determined by bicinchoninic acid (BCA) protein assay kit (Interchim). Transfection efficiency was expressed as relative light units (RLU) per mg of protein (RLU/mg protein).

2.7 | RNA extraction and RT-qPCR

RNA in transfected cells was extracted with NucleoSpin RNA Plus (Macherey-Nagel). qRT-PCR was carried out using QuantiTect SYBR Green RT-PCR (Qiagen) according to the manufacturer's instructions. Data were analyzed with $2^{-\Delta\Delta C_t}$ methods using GAPDH as a loading control. The following primers were used: 5'-TGGGACCAGAG GAAGAAGC-3' (forward) and 5'-ATGCCCACTCCTTG ATGATTC-3' (reverse) for TRAIL; 5'-GTGGACCTGAC CTGCCGTCT-3' (forward) and 5'-GGAGGAGTGGGTGT CGCTGT-3' (reverse) for GAPDH (Zhang et al., 2012); 5'-GGAAGCGAATCAATGGACTCTGG-3' (forward) and 5'-GCATCGACATCTGTACCAGACC-3' (reverse) for caspase 3 (Origene). Reverse transcription was performed at 50°C for 30 min, enzyme was activated at 95°C for 15 min prior to 40 cycles of denaturation at 94°C for 15 s, annealing at 54.5°C for 30 s, and extension at 72°C for 30 s.

2.8 | MTT assay

The cisplatin-resistance level (2.5–20 μ M) (Comsa et al., 2018) of CR-Caov3 and the cellular growth inhibitory rate of different treatments were determined by MTT-Cell proliferation kit I (Sigma Aldrich). Z-VAD(OMe)-FMK (Medchemexpress) was used at 100 μ M (Wang et al., 2012) to determine if the anti-proliferative effect was caspase-dependent. After transfection and/or cisplatin treatment and/or pan-caspase inhibitor Z-VAD(OMe)-FMK (Z-vad) incubation, the MTT labeling reagent (terminal concentration of 0.5 mg/mL) was incubated with the cells for 4 h. Afterward, solubilization solution (100 μ L) was added to each well. Subsequently, the plate was incubated overnight at 37°C and 5% CO₂. The absorbance was measured at 570 nm using a reference wavelength of 690 nm. Cell

viability was determined relative to untreated control cells. Afterwards, the viability of cells transfected with TRAIL-encoding plasmid was compared to those transfected with control plasmid pGM144.

2.9 | Flow cytometry analysis of Annexin/PI apoptosis assay and DR4, DR5 expressions

The apoptosis rate was analyzed using the Annexin V/PI apoptosis detection kit (Sigma-Aldrich) per the supplier's instructions. Data were acquired on a BD Accuri C6 flow cytometer and analyzed with BD Accuri C6 software.

(FITC)-conjugated anti-human DR4 and DR5 antibodies (Santa Cruz Biotechnology) were used at 4 µg/mL to examine the expression of DR in various cell lines with or without cisplatin treatment.

2.10 | Wound healing assay

Cells were cultured in 96-well plates until reaching confluent monolayer. After transfection and/or cisplatin incubation time, cells were washed with PBS before and after the similar sized wounds were made by using 10-µL pipette tips. Cells were then cultured in serum-free medium to minimize cell proliferation. The wound gaps were photographed at 0 and 24 h, and the area of wound surface was measured using ImageJ software with the plugin developed by Suarez-Arnedo et al. (2020). The migration rate of each sample was calculated with the following formula:

$$\text{Migration rate (\%)} = \frac{(\text{Wound area at 0 h}) - (\text{Wound area at 24 h})}{\text{Wound area at 0 h}} \times 100$$

2.11 | Caspase activity assay

Considering that caspase-8 is the first and most important caspase activated during TRAIL's signaling pathway (Dickens et al., 2012), its activity in cancer cells was determined by the caspase activity fluorescence assay (Abcam) following different treatments according to the manufacturer's protocols. The fluorescence was measured using a multimode microplate reader (Mithras² LB 943).

2.12 | Statistical analysis

Statistical analysis was carried out using GraphPad Prism 7.0. Results are presented as mean ± standard deviation.

Differences between groups were analyzed using the Student's *t*-test. *p* < .05 was considered significant.

3 | RESULTS

3.1 | BSV163/DOPE physicochemical characterization

Physicochemical parameters have significant influences on transfection efficiency. It is generally known that smaller nanoparticles will present a higher extent of cellular uptake. Resnier et al. (2013) suggested that the delivery system should range between 20 and 200 nm considering the potential elimination by renal clearance and by innate immune system with particle of size less than 20 nm and superior to 200 nm respectively. As reported in Figure 1a, the hydrodynamic diameters of both BSV163/DOPE and its related complexes with pVAX2-TRAIL are found within this range. The addition of pDNA at various CRs slightly increased the lipoplexes diameter from 101.32 ± 4.75 nm to around 130 nm and slightly decreased the zeta potential from +69.88 ± 3.34 to around +50 mV. The polydispersity index of BSV163/DOPE-pVAX2-TRAIL complexes was around 0.2–0.3 indicating a low size dispersion of these lipoplexes. These parameters are in good agreement with our previous report (Ghanem et al., 2023). In addition, BSV163/DOPE is capable of efficiently encapsulate and protect pVAX2-TRAIL as shown by the band retardation from CR2 to CR5 (Figure 1b, –ds). As the CR increased, the plasmid encapsulation efficiency also increased. Indeed, at CR5, the complexation was nearly complete. Furthermore, when dextran sulfate was added to each complex, a same band as non-encapsulated pVAX2-TRAIL, was observed (Figure 1b, +ds). These results proved that BSV163/DOPE can entrap and protect the nucleic acids cargo and allow the efficient release of pDNA. The pDNA complexation and relaxation capabilities were not pVAX2-TRAIL-specific as similar results were also achieved in our preceding publication using a different plasmid (Ghanem et al., 2023).

3.2 | Generation of cisplatin-resistant ovarian cancer cell line

Cisplatin is a well-known cytotoxic drug that kills ovarian cancer cells by damaging DNA. Cisplatin-resistant Caov3 (CR-Caov3) cell line was gradually selected from the parental (Caov3) cell line by constant exposure to gradually increasing concentrations of cisplatin over 6 months. Afterward, the cisplatin sensitivity of different cell lines was determined by MTT assay as described above. The results in Figure 2 and Figure S1 demonstrated that CR-Caov3 cells were more resistant to the killing effect of cisplatin at

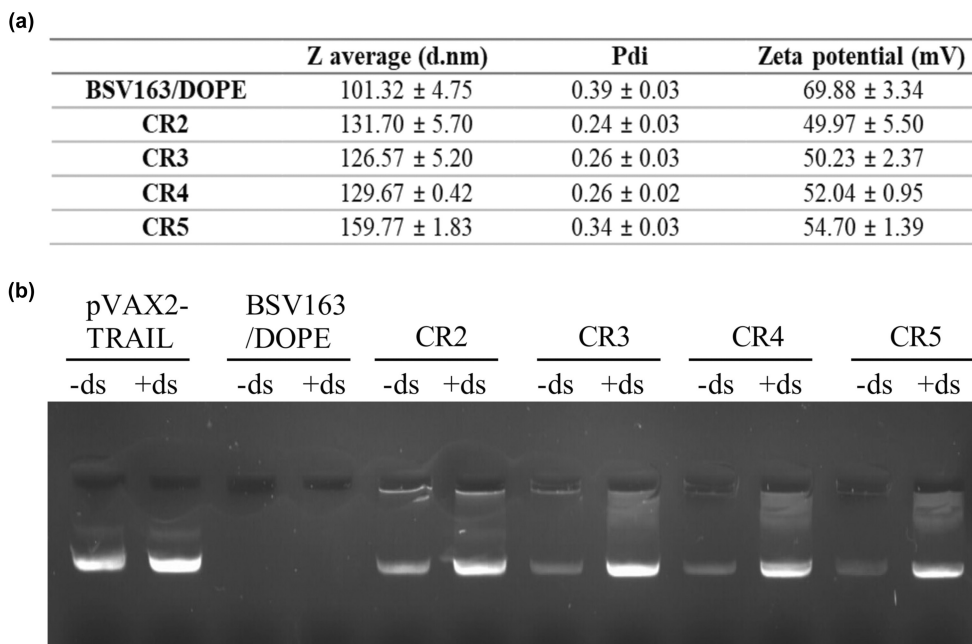


FIGURE 1 (a) Z-average (d.nm), polydispersity index (Pdi) and Zeta potential (mV) of BSV163/DOPE and its related complexes with pVAX2-TRAIL at different charge ratios (CRs 2–5). Three measurements were performed and mean values ± SD were reported. (b) Electrophoresis of pVAX2-TRAIL, BSV163/DOPE, or BSV163/DOPE-pVAX2-TRAIL complexes at different charge ratios (CRs 2–5), in the absence (–ds) or presence (+ds) of dextran sulfate.

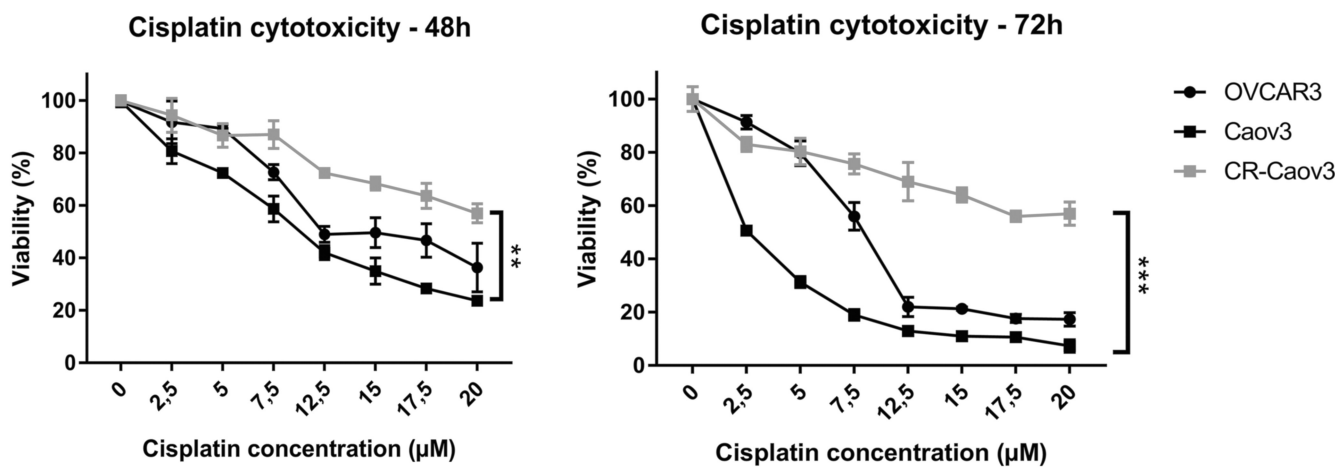


FIGURE 2 Response of cisplatin-resistant and -sensitive ovarian cancer cell lines to cisplatin treatment: OVCAR3, Caov3, and CR-Caov3 cells were exposed to a range of cisplatin concentrations (2.5–20 µM). After 48 or 72 h of incubation, cell viability was evaluated by MTT assay. Differences in cellular viability were analyzed using the Student’s *t*-test. *Corresponding to a *p* value <.05; **corresponding to a *p* value <.01; ***corresponding to a *p* value <.001.

various concentrations after 48 or 72 h of incubation than the original line Caov3 and the cisplatin-sensitive line OVCAR3.

3.3 | Transfection efficiency versus cytotoxicity evaluation

The cationic lipid BSV163, conjugated with DOPE (1:1, molar ratio), was exploited to transfer pGM144 to A549

and 16HBE cell lines and was proved to be more efficient than branched polyethylenimine in the preceding works (Bouraoui et al., 2020; Ghanem et al., 2023). These data prompted us to investigate if this would be translatable to different ovarian cancer cell lines. Indeed, as demonstrated in Figure 3 (left panels), luciferase activity was observed following transfection with pGM144 encapsulated by BSV163/DOPE. In general, transfection efficiencies at different charge ratios (CRs 2–5) seemed to be related to cytotoxicity in a way that the greater charge

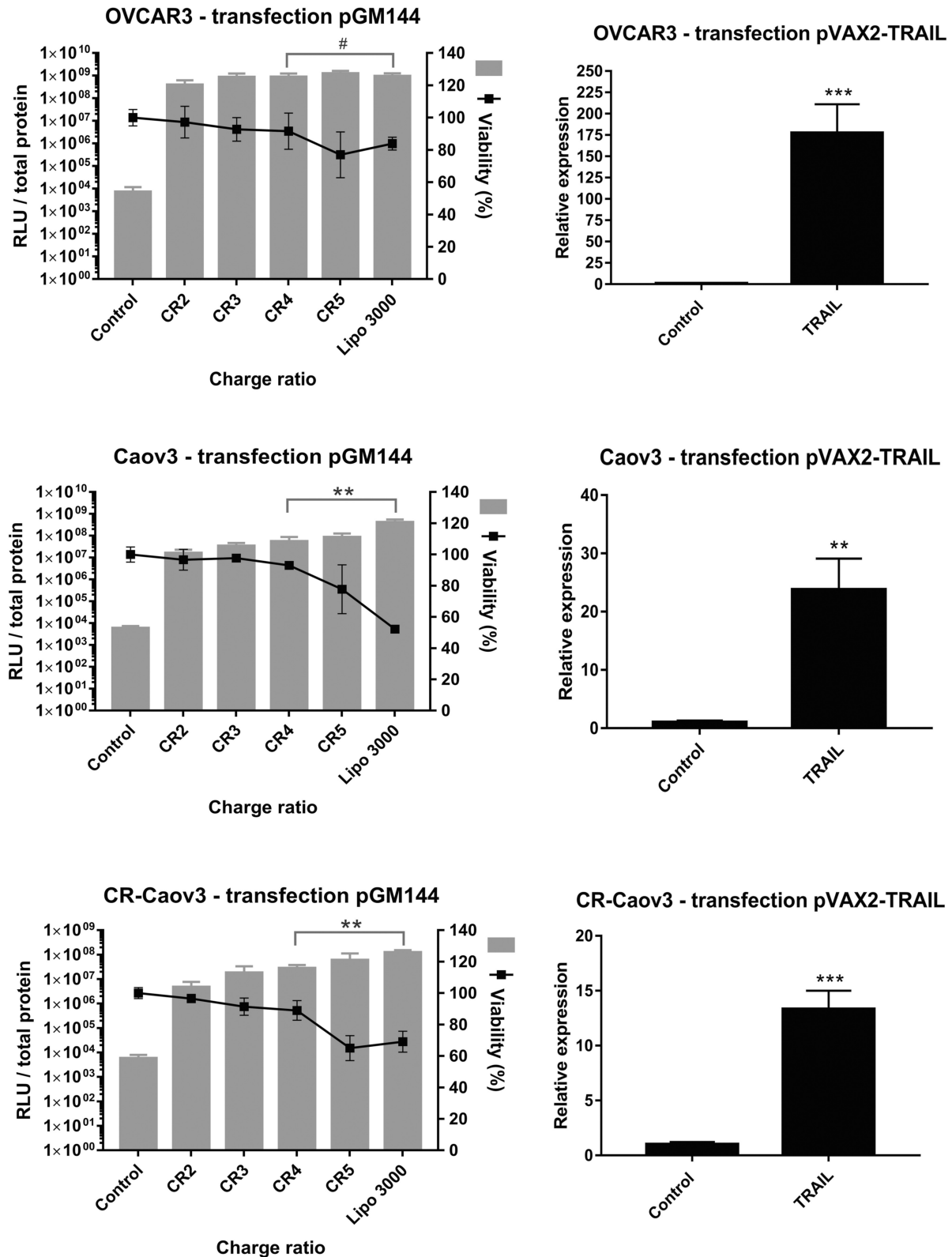


FIGURE 3 Transfection efficiency of BSV163/DOPE in cisplatin-resistant and -sensitive ovarian cancer cell lines in the absence of cisplatin. In the left panels, after 48 h of transfection using different charge ratios (CR), BSV163/DOPE/pGM144-mediated luciferase gene expression and cell viability were determined as mentioned above. In the right panels, BSV163/DOPE/pVAX2-TRAIL-mediated TRAIL gene expression at the mRNA level was verified by qRT-PCR after 24 h of transfection using CR4. Control, untreated cells; CRs 2–5, charge ratio; Lipo 3000, Lipofectamine 3000; RLU, relative light units. Differences between groups were analyzed using the Student's *t*-test. *Corresponding to a *p* value < .05; **corresponding to a *p* value < .01; ***corresponding to a *p* value < .001; # corresponding to a *p* value > .05 (non-significant).

ratio would result in higher gene delivery capacity and also lower cell viability. The transfection efficiency of BSV163/DOPE at CR4 was comparable to the commercial vector Lipofectamine 3000 in OVCAR3 cells and was lower in Caov3 and CR-Caov3 cells. However, the application of BSV163/DOPE was significantly safer in terms of cellular viability (Figure 3, left panels). In addition, BSV163/DOPE has also demonstrated its safety and efficacy *in vivo* in our previous report (Ghanem et al., 2023) while Lipofectamine 3000 has only been optimized for *in vitro* study. The fall of cell viability following transfection employing the CR5 also suggested the CR4 to be the best compromise between transfection efficiency and toxicity which was then chosen for further experiments. Using CR4, we transfected different cell lines with TRAIL-encoding plasmid pVAX2-TRAIL and confirmed the expression of TRAIL mRNA via qRT-PCR assay in cisplatin-sensitive ovarian cancer cells as well as its resistant analogs (Figure 3, right panels).

3.4 | Cell viability assay

Following different treatments, the cellular viability was examined by MTT assay (Figure 4a,b, left panels). With cisplatin-sensitive OVCAR3 and Caov3 cell lines, significant decreases in cellular viability were observed in cells treated with cisplatin compared to control cells and in cells transfected with TRAIL-encoding plasmid compared to those transfected with control plasmid pGM144. Interestingly, cell lethality of the combined treatment TRAIL transfection + cisplatin (50% in Caov3) was greater than that of each treatment alone (TRAIL transfection or cisplatin) which indicated the combinatory killing effect between these therapeutic agents (Figure 4a,b, left panels). Most importantly, such a synergistic effect was also observed in the CR-Caov3 cell line (nearly 30%) despite the modest killing effect of cisplatin in these cells (only 3%) compared to other sensitive lines (Figure 4c, left panels).

To determine whether or not the anti-proliferative effect was caspase-dependent, the pan-caspase inhibitor Z-vad (100 μ M) was employed following different treatments. Z-vad blocked caspases activation and therefore remarkably restrained cell death after TRAIL transfection, cisplatin incubation or combined treatment in all cell lines (Figure S2a). However, inefficient transgene expression could also lead to decreased anti-cancer effect. To ensure that Z-vad did not interfere with transfection process, all cell lines were transfected with luciferase-coding pGM144 and then incubated with the same Z-vad concentration. We confirmed that the transfection efficacy was not affected by Z-vad treatment in

all cell lines (Figure S2b). These results indicated that TRAIL transfection and/or cisplatin cause caspases cascade to initiate cell death.

3.5 | Measurement of caspases activity

It has been well established that the activation of caspase 8 is crucial for TRAIL-induced cell death signaling pathways (Alizadeh Zeinabad & Szegezdi, 2022). Based on this premise, we measured caspase 8 activity in cisplatin-sensitive and -resistant ovarian cancer cells following different treatments. Consistent with the results of the viability assay, we also demonstrated that the combined treatment activated caspase 8 more efficiently compared to control cells and each treatment alone (Figure 4a–c, right panels). Caspases act in cascade to mediate apoptosis and at the end, activated caspase 3 is responsible for killing the cells (Nagata, 2018). Therefore, the caspase 3 overexpression after various treatments was verified by RT-pPCR. The results were in agreement with caspase 8 activity. The combined treatment was also the most efficient activator of caspase 3 (Figure S2c).

3.6 | Apoptosis rate following different treatments

Since synergistic treatment of cisplatin and TRAIL-transfection affected cellular growth rates effectively, we next sought to investigate the effect of such treatment on apoptosis in different cell lines using flow cytometry. Of note was the evident enhancement in the apoptosis rate associated with the combined treatment in CR-Caov3 cells (nearly 35%) in comparison with TRAIL transfection (17%) or cisplatin treatment alone (6%) (Figure 5c). Similar results were noted in cisplatin-sensitive cell lines with the most significant increase in apoptosis rate induced by the combination of TRAIL transfection plus cisplatin (nearly 50% and 55% with Caov3 and OVCAR3 cells, respectively) (Figure 5a,b). The results of apoptosis and caspase assay confirmed that TRAIL-BSV163/DOPE transfection acted synergistically with cisplatin to induce apoptosis in cisplatin-sensitive and -resistant ovarian cancer cells.

3.7 | DR expression

The binding of TRAIL to its DR4 and DR5 is the first step to induce cell death signaling (Alizadeh Zeinabad & Szegezdi, 2022). Therefore, the presence of these receptors

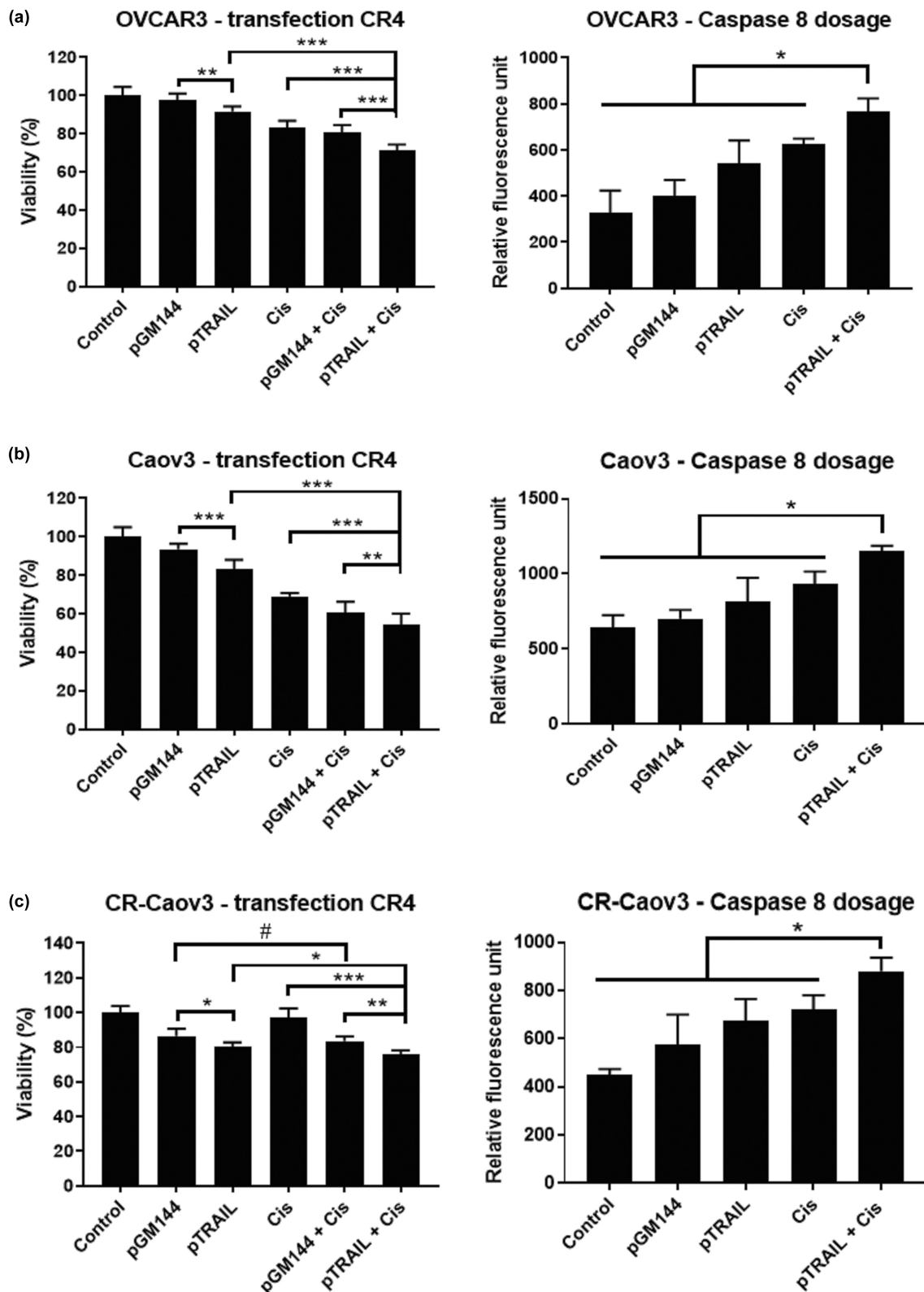


FIGURE 4 Biologic activity of BSV163/DOPE/pVAX2-TRAIL transfection in combination with cisplatin treatment in cisplatin-sensitive (a, b) and -resistant (c) ovarian cancer cell lines. BSV163/DOPE/pVAX2-TRAIL transfection (48 h) acted synergistically with cisplatin treatment (7.5 μ M) to induce cell death (measure by MTT assay, left panels) and caspase-8 activation (measured by caspase-8 activity assay, right panels). Cis, cisplatin treatment; Control, untreated cells; GM144, control transfection using pGM144; TRAIL, transfection using pVAX2-TRAIL. Differences between groups were analyzed using the Student's *t*-test. *Corresponding to a *p* value <.05; **corresponding to a *p* value <.01; ***corresponding to a *p* value <.001; #corresponding to a *p* value >.05 (non-significant).

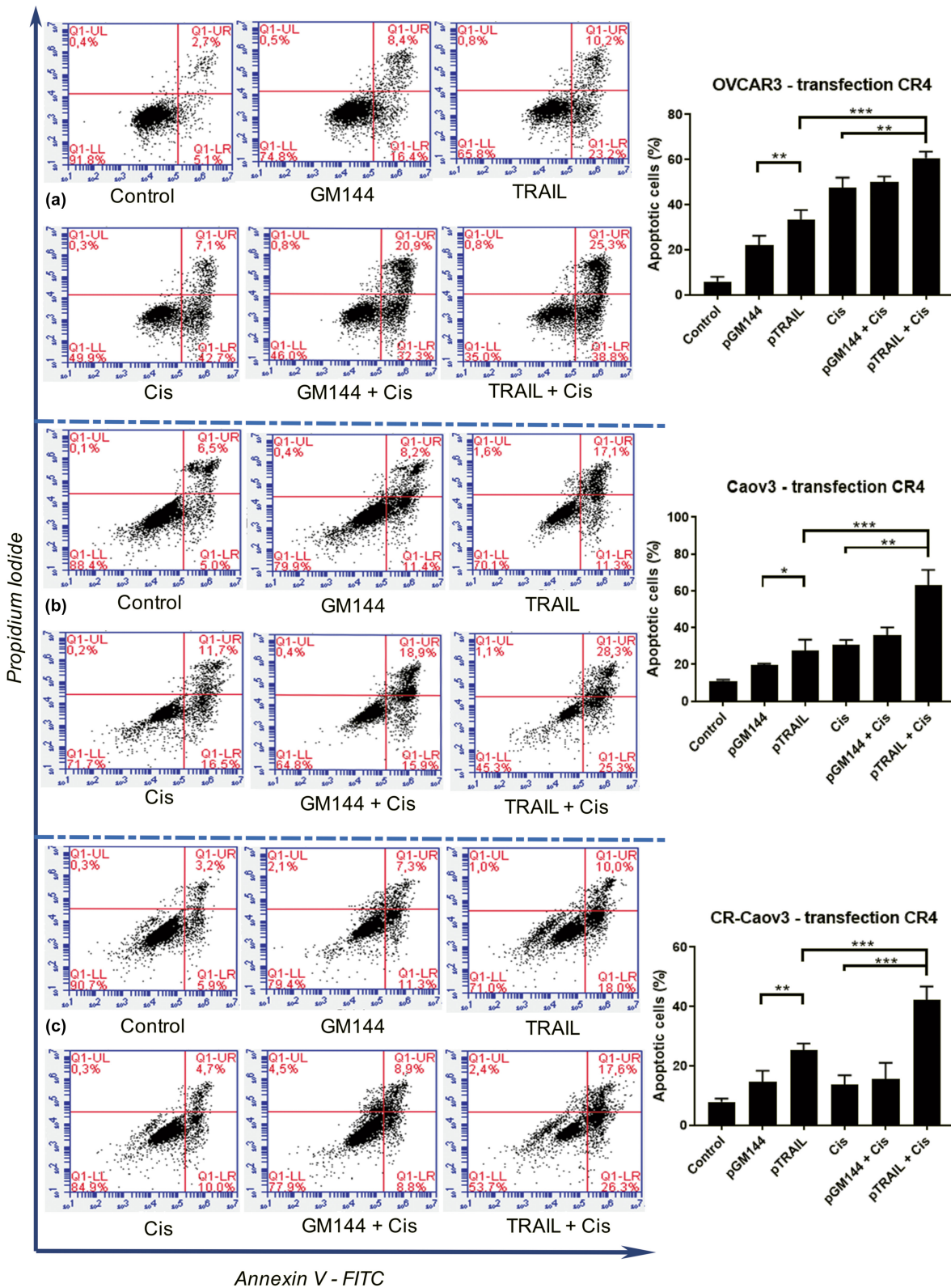


FIGURE 5 Cisplatin synergistic effects on BSV163/DOPE/pVAX-TRAIL-induced apoptosis following 48 h of transfection in (a) OVCAR3 cells, (b) Caov3 cells, and (c) CR-Caov3 cells. Cis, cisplatin treatment; Control, untreated cells; GM144, control transfection using pGM144; TRAIL, transfection using pVAX2-TRAIL. Differences between groups were analyzed using the Student's *t*-test. *Corresponding to a *p* value <.05; **corresponding to a *p* value <.01; ***corresponding to a *p* value <.001. Representative images of the flow cytometry analysis are presented in Figures S4 and S5.

on the cell surface indicates cancer cells' susceptibility to TRAIL death signaling. To investigate DR expression, cisplatin-sensitive cell lines OVCAR3, Caov3 and its cisplatin-resistant analog CR-Caov3 were probed with FITC-conjugated anti-DR4 and -DR5 antibodies. As demonstrated in [Figure 6](#), all three cell lines presented DR4 and DR5 on the cell surface. Notably, the expression of DR was enhanced following cisplatin treatment in all cell lines, including cisplatin-resistant CR-Caov3 cells.

3.8 | Wound healing assay

The impact of various treatments on cancer cell migration was determined using the wound healing assay (Cory, 2011; Liu et al., 2017; Wu et al., 2019). Our results illustrated that the combined treatment inhibited migration of CR-Caov3 cells more effectively in comparison with TRAIL transfection or cisplatin alone as well as the control groups after 24 h ([Figure S3](#)).

4 | DISCUSSION

Even with the development of gene delivery techniques targeting malignant cells in recent years and the promising characteristics of TRAIL as an anti-cancer agent, studies employing TRAIL gene therapy in the treatment of ovarian cancer have been relatively rare and mostly based on viral systems (Li et al., 2012). Virus-based vectors have high transfection efficiencies but also drawbacks including the risk of insertional mutagenesis and immune-mediated rejection which can be dangerous for cancer patients (Butt et al., 2022). The nano-based bioinspired cationic lipid vector BSV163/DOPE is more cost-effective, has been thoroughly characterized and has recently demonstrated effectiveness and safety in an *in vivo* study (Ghanem et al., 2023). These findings fueled investigation into BSV163/DOPE-based application in cancer gene therapy. Indeed, it was obvious in the present work that BSV163/DOPE can be exploited to effectively transfect two cellular models that represent properly high-grade serous ovarian cancer (Domcke et al., 2013) as well as its cisplatin-resistant analogs.

The resistance to platinum salts in patients suffering from recurrent or refractory diseases is one of the major problems in ovarian cancer management. In the present study, we have observed the synergistic effect of BSV163/DOPE-pVAX2-TRAIL transfection and cisplatin treatment in inhibiting the growth rate of both cisplatin-sensitive and -resistant ovarian cancer cell lines ([Figure 4](#)). These results showed that cisplatin-resistant CR-Caov3 cells did not have cross-resistance to TRAIL and implied that

BSV163/DOPE-pVAX2-TRAIL transfection could act synergistically with conventional chemotherapy resulting in ameliorated growth-inhibitory effect in sensitive and refractory ovarian cancer.

It has been well established that ovarian cancer cell lines and patients' specimens express on the cellular surface at least one of the two TRAIL death receptors (Duiker et al., 2010). These expressions are enhanced after chemotherapy (Duiker et al., 2011). Our results are in good agreement with these studies while adding similar findings to the cisplatin-resistant CR-Caov3 cells ([Figure 6](#)). These observations could explain partly the mechanisms underlying the combinatorial effect of BSV163/DOPE-pVAX2-TRAIL transfection and cisplatin treatment. Indeed, TRAIL binding leads to death receptor (DR) oligomerization which recruits the adapter Fas-associated death domain protein (FADD) via death domain interaction between DR and FADD. FADD in turn recruits the pro-caspase-8 through its N-terminal death effector domain. The assemblage of DR, FADD and pro-caspase-8 is known as the death-inducing signaling complex (DISC) (Alizadeh Zeinabad & Szegezdi, 2022). The effective oligomerization of DR and FADD is of particular importance in activating pro-caspase-8 and transducing an efficient death signal (Muppidi et al., 2006; Yan et al., 2013). Actually, the inefficient cleavage and activation of pro-caspase-8 favor cell survival instead of cell death which could be the downside of TRAIL signaling in terms of cancer treatment (Hughes et al., 2009). As demonstrated by our results, the cisplatin-mediated upregulation of DR in the cell surface succeeded in increasing the DR-induced caspase 8 activation upon TRAIL ligation ([Figures 4 and 6](#)). Triggering caspase 8 activation initiated a caspase cascade and at the end, caspase 3, leading ultimately to an increase of apoptosis rate in cisplatin-sensitive and -resistant ovarian cancer cell lines ([Figure 5; Figure S2](#)). These observations fitted quite well with the results of the viability assay. We also demonstrated that the combined treatment inhibited efficiently cisplatin-resistant ovarian cancer cells migration ([Figure S3](#)). Altogether, these data suggested the potential of TRAIL gene therapy to re-sensitize ovarian cancer cells that have become resistant after long-term exposure to cisplatin.

One of the most promising characteristics of TRAIL as an anti-cancer agent is its biocompatibility with normal healthy cells. However, potential hepatotoxicity has been reported upon TRAIL treatment using its recombinant form or agonistic antibodies targeting its receptor (Papadopoulos et al., 2015). In addition, the recombinant form of TRAIL has a relatively short half-life *in vivo* (Zhong et al., 2019). These data greatly dampened its clinical application which explains the development of TRAIL gene therapy as an alternative in order to overcome these limitations (Jiang et al., 2016). TRAIL gene therapy has the potential to improve the

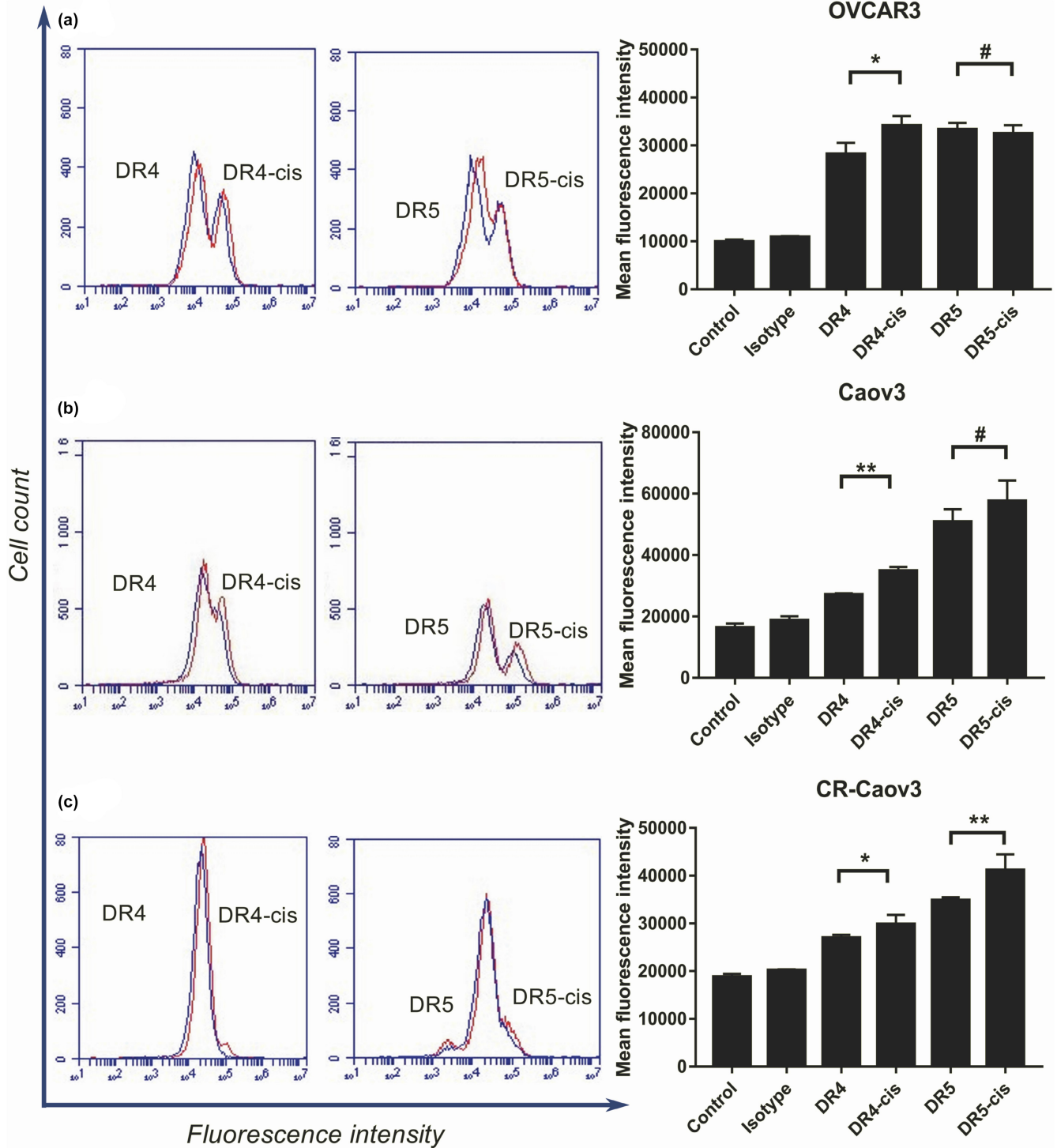


FIGURE 6 Flow cytometry analysis of the cell surface expression of DR4 and DR5 in cisplatin-sensitive (a,b) and -resistant (c) ovarian cancer lines after being treated with cisplatin (7.5 μ M) for 24h. cis, cisplatin treatment; Control, untreated cells. Differences between groups were analyzed using the Student's *t*-test. *Corresponding to a *p* value <.05; **corresponding to a *p* value <.01; # corresponding to a *p* value >.05 (non-significant). Representative images of the flow cytometry analysis are presented in [Figure S6](#).

stability and short half-life of TRAIL and to deliver TRAIL-encoding DNA into tumor cells locally. In the field of ovarian cancer, Yin et al. (2016) have recently demonstrated the anti-cancer effect of adipose-derived mesenchymal stem

cells transfected by TRAIL-incorporating magnetic core-shell nanoparticles. Their results also demonstrated the sensitivity to TRAIL gene therapy of cisplatin-sensitive ovarian cancer cell line (A2780).

The main drawback of synthetic vectors lies in their relatively low transfection efficiency compared to that of viral vectors. However, considering the safety in vivo of lipid vectors, multiple injections can be carried out to address this issue. Regarding BSV163/DOPE, our next step consists of integrating acid folic into our formulation knowing that folate receptor α is overexpressed in the majority of ovarian cancer (Mornet et al., 2013; Scarranti et al., 2020). In fact, it has already been shown that compared to non-modified liposomes, folate-integrated lipoplexes can enhance transfection efficiency and specifically target malignant cells (He et al., 2016). In addition, ovarian cancer-specific promoters such as telomerase reverse transcriptase (hTERT) promoter will also be used to drive the expression of our transgene to constitute a double targeting strategy. This should ensure an ameliorated cancer specificity while minimizing undesirable effects on normal cells.

5 | CONCLUSIONS

In summary, this preliminary study demonstrated the applicability of an innovative bioinspired lipid-based vector BSV163/DOPE in ovarian cancer studies. Cellular transfection of the TRAIL gene using BSV163/DOPE in combination with cisplatin treatment successfully inhibited cell growth and triggered apoptosis in cisplatin-sensitive ovarian cancer cells as well as its resistant analogs. These results should encourage the tuning of BSV163/DOPE to further enhance transfection efficacy and targeting ability to fit the future desired application and move into in vivo studies.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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