

Efficient inhibition of intraperitoneal human ovarian cancer growth and prolonged survival by gene transfer of vesicular stomatitis virus matrix protein in nude mice

Xiaojuan Lin^a, Xiancheng Chen^a, Yuquan Wei^b, Jumei Zhao^b,
Linyu Fan^b, Yanjun Wen^b, Hongbo Wu^b, Xia Zhao^{a,*}

^a Department of Gynecology and Obstetrics, Second West China Hospital, Sichuan University, Chengdu, Sichuan, 610041, The People's Republic of China

^b National Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, 610041, The People's Republic of China

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Abstract

Objective. Vesicular stomatitis virus (VSV) matrix protein (MP) has been reported to be capable of inducing apoptosis *in vitro* in the absence of other viral components. In the present study, the antitumor effect of a recombinant plasmid encoding VSVMP on human ovarian cancer and its apoptosis-inducing efficacy *in vivo* were further investigated.

Methods. The recombinant plasmid DNA carrying VSVMP-cDNA (VSVMP-p) was constructed. SKOV3 ovarian cancer cells were transfected with VSVMP-p and examined for apoptosis by Hoechst 33258 staining and flow cytometric analysis. For *in vivo* study, intraperitoneal ovarian carcinomatosis models in nude mice were established and randomly assigned into four groups to receive six twice-weekly i.p. administrations of VSVMP-p/liposome complexes, empty plasmid/liposome complexes, liposome alone or 0.9% NaCl solution, respectively. The weight of intraperitoneal carcinomatosis and the survival were monitored. Tumor tissues were inspected for apoptosis by TUNEL and Hoechst-33258 assay.

Results. Plentiful apoptosis were observed in SKOV3 cells transfected with VSVMP-p. VSVMP-p reduced intraperitoneal tumor weight by about ~90% compared with control agents ($p < 0.01$) and significantly prolonged the survival of tumor-bearing mice ($p < 0.05$), with *in vivo* apoptosis index of $12.6 \pm 2.7\%$ which was much higher than that of control groups ($< 4\%$) ($p < 0.05$). Interestingly, this antitumor effect was accompanied by a noticeable NK cell accumulation. The treatment with VSVMP-p was devoid of any conspicuous toxicity.

Conclusions. These observations suggest that VSVMP-p have strong antitumor effects by inducing apoptosis and possibly NK cell-mediated tumor resistance mechanisms, and it may be a potentially effective novel therapy against human ovarian cancer.

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Keywords: Vesicular stomatitis virus matrix protein (VSVMP); Apoptosis; Lymphocyte accumulation; Ovarian cancer; Cationic liposome

Introduction

Vesicular stomatitis virus (VSV), a negative-stranded RNA rhabdovirus with a single nonsegmented genome that contains only five protein-encoding genes (N, P, M, G and L), can preferentially replicate in immortalized and malignant cells and eventually induce apoptosis [1–4]. Studies by a number of researchers have also confirmed that VSV can suppress the growth of various tumors when introduced intratumorally or

intravenously in tumor-bearing animal models [3–7]. The intriguing questions for researchers are which viral components play an important role in inducing apoptosis in VSV-infected cells and whether they still possess this function when used alone to avoid potential biohazard of virus infection. Recent studies have demonstrated that it is the matrix protein (MP), a structural component of the virion, that causes considerable cytopathogenesis of VSV in the absence of other viral components, including the inhibition of host gene expression, the elicitation of cell rounding and the induction of apoptosis [8–13]. Kopecky and Lyles [14] further analyzed the cause and effect relationships of the three cellular effects of MP and

* Corresponding author. Fax: +86 028 85250731.

E-mail address: xia-zhao@126.com (X. Zhao).

concluded that inhibition of host gene expression caused apoptosis, which in turn led to the induction of cell rounding.

The potent ability of MP to induce apoptosis *in vitro* raises such hypotheses as “Whether MP can elicit apoptosis of tumor cells *in vivo*” and “Can it be a promising agent for malignant disease”. To test these hypotheses, we constructed a recombinant plasmid DNA carrying VSVMP-cDNA (VSVMP-p), administered it in a few tumor models using cationic liposome as a gene delivery system (including Lewis lung carcinoma and CT26 mouse colon adenocarcinoma *in vitro*, and s.c. Lewis lung carcinoma model and s.c. MethA fibrosarcoma model *in vivo*) and found that it had potent antitumor activity (Zhao JM et al., manuscript submitted for publication).

Ovarian cancer accounts for 4% of all cancers in women and is the principal cause of death among the gynecological malignancies [15]. Aggressive surgical debulking along with multiagent cytotoxic chemotherapy has therapeutically benefited patients with advanced, epithelial ovarian cancer and newly developed biotherapies along with rarely used radiation therapy may be helpful supplements. However, the mortality rate of ovarian cancer remains exceptionally high [16,17]. This dismal status quo reflects a current lack of available, more effective treatments. Considering that gene transfer of VSVMP was able to induce apoptosis *in vitro* and inhibit the growth of a few tumors *in vivo*, the present study is designed to evaluate the antitumor effect of VSVMP-p as an experimental treatment for ovarian cancer. In this study, VSVMP-p proved able to effectively induce apoptosis of SKOV3 ovarian cancer cells *in vitro*; furthermore, intraperitoneal administration of VSVMP-p/liposome complexes demonstrated noticeable efficacy against peritoneal carcinomatosis model in nude mice, resulting in suppressed tumor growth and prolonged survival owing to the capacity of VSVMP-p to induce apoptosis and possible NK cell accumulation.

Materials and methods

Cell line

The human ovarian serous cystadenocarcinoma cell line SKOV3 was obtained from American Type Culture Collection (Rockville, Maryland) and cultured in RPMI 1640 tissue culture medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine and 0.1 mg/ml amikacin. Cells were incubated in a humidified atmosphere containing 5% CO₂ at 37°C and passaged every 5 days at a split ratio of 1:4 using trypsin.

Plasmid construction and liposome preparation

pcDNA3.1 plasmid (Invitrogen, San Diego, CA) expressing wild-type VSVMP, named VSVMP-p, was constructed in our laboratory. Briefly, cDNA clone encoding VSVMP was PCR amplified with the upstream primer CGC GGA TCC ATC ATG AGT TCC TTA AAG AAG and the downstream primer CGG AAT TCT CAT TTG AAG TGG CTG ATA GAA TCC. Then it was digested with *Bam*H1/*Eco*R1 and inserted into pcDNA3.1 digested with *Bam*H1/*Eco*R1 to generate VSVMP-p. As a control, pcDNA3.1 plasmid without VSVMP-cDNA was used as an empty vector (e-p). Colonies of *Escherichia coli* containing VSVMP-p or e-p were cultured in Luria-Bertani broth containing 100 µg of ampicillin/ml. Large-scale plasmid DNA was purified using an EndoFree Plasmid Giga kit (Qiagen, Chatsworth, CA). The DNA was eventually dissolved in sterile endotoxin-free water at a concentration of 5.0 mg/ml, stored at –20°C before use. The

recombinant VSVMP-p was confirmed by restriction digestion and DNA sequencing.

The lipids DOTAP (dioleoyl trimethylammonium propane; Avanti Polar Lipids Inc., AL) and DOPE (dioleoyl phosphatidylethanolamine; Avanti Polar Lipids Inc., AL) (1:1 molar ratio) were dissolved in chloroform supplemented with methanol (3:1 volume ratio). The resulting mixture was dried in a rotary 100-ml round-bottomed flask and organic solvent was further removed under vacuum for 2 h. The lipid film was hydrated in appropriate amount of sterile water to yield a final concentration of 2.5 mg/ml. Then the liposome solution was vortexed for 1 min, sonicated for 10 min to form small unilamellar vesicles and stored at 4°C.

Apoptosis assay *in vitro*

In order to determine the optimal plasmid:liposome ratio (µg/µg) for efficient gene delivery, a series of experiments *in vitro* with different plasmid:liposome ratios transfecting SKOV3 cells were carried out. The plasmid used herein was the recombinant pcDNA3.1 plasmid encoding for the green fluorescent protein (GFP) and a maximum expression was obtained when 5 µg liposome/µg DNA was used (data not shown).

To test the effect of VSVMP-p *in vitro*, aliquots of 1×10^5 SKOV3 cells were grown in each well of 6-well plates in triplicate and incubated for 72 h to 80% confluence. DNA (VSVMP-p or e-p)/liposome complexes were prepared in RPMI 1640 medium, which contained 2 µg DNA and 10 µg liposome, and left at room temperature for 30 min. In addition, 10 µg liposome alone and medium alone were also used as control agents. The cells were incubated with the above agents for 6 h, rinsed three times with PBS and then 1.5 ml of RPMI 1640 supplemented with FCS was added to each well, with a continued incubation for an additional 48 h. Apoptosis was observed by Hoechst 33258 staining (Apoptosis-Hoechst staining kit; Beyotime Biotechnology, Chin). Briefly, cells were immersed in 0.5 ml of methanol for 15 min, followed by rinse with PBS twice. Then cells were stained with 1 µg/ml Hoechst 33258 compounds in a dark chamber at room temperature for 10 min and rinsed twice in PBS again. Cells were analyzed by fluorescence microscopy using excitation 348 nm/emission 480 nm wavelengths. The apoptotic cells are featured as pyknotic and fragmented nuclei emitting intense fluorescence [18].

In addition, quantitative evaluation of cellular apoptosis was performed by flow cytometric analysis using annexin V-propidium iodide (PI) double staining method. Briefly, after processed in 6-well plates as described above, the floated cells were discarded and the attached cells were trypsinized and thereafter washed twice with cold PBS. Cells were resuspended in prediluted binding buffer and stained with FITC-annexin V (BD Pharmingen, CA) for 10 min in the dark at room temperature. Then they were washed and resuspended in binding buffer. PI (1 µg/ml) was added, and the mixtures were analyzed by flow cytometry immediately.

Animals and the establishment of peritoneal carcinomatosis model

Female athymic BALB/c nude mice, 6 to 8 weeks old, were housed in autoclaved microisolator cages in an air-filtered laminar flow cabinet and were given food and water *ad libitum*. All procedures were performed under sterile conditions in a laminar flow hood. This animal experiment was approved by the Institutional Animal Care and Use Committee and was in compliance with all regulatory guidelines.

SKOV3 cell suspension (5×10^6 cells in 100 µl of RPMI 1640 medium) was severally injected s.c. in the backs of five nude mice. The purpose of developing s.c. tumors was to generate histologically intact tumors for i.p. implantation. When the diameter of tumors reached up to about 1 cm, tumors were collected, excised free of necrotic areas and then minced with scissors and tweezers into small particles with a diameter of less than 1 mm. Sufficient RPMI 1640 medium was added into these tumor particles and the total volume of the mixture was made at 16 ml. Then 32 nude mice were respectively inoculated i.p. with 0.5 ml of the mixture in the right lower quadrant with a 14-gauge needle. In a previous study performed in our laboratory, consecutive monitor of the mice undergoing the above procedure revealed that the i.p. inoculation resulted in extensive dissemination of intraperitoneal carcinomatosis and macroscopic or

microscopic tumor nodules could be observed 7 days after inoculation. Therefore, the following therapy studies were initiated 7 days after the injection of tumor particles.

Therapy studies

Before i.p. administration, the DNA/liposome complexes were prepared by adding DNA solution to liposome solution at a ratio of 1 μg DNA to 5 μg liposome and the mixture was gently pipetted up and down twice. Then the mixture was incubated at room temperature for 30 min.

Seven days after i.p. tumor inoculation, twelve of these mice were randomly assigned into one of the following four groups ($n=3$): (a) mice treated with 50 μg VSVMP-p/250 μg liposome complexes, (b) mice treated with 50 μg e-p/250 μg liposome complexes, (c) mice treated with 250 μg liposome alone (volume=100 μl) and (d) mice treated with 100 μl of 0.9% NaCl solution (NS). They received six twice-weekly i.p. administrations and were monitored on a daily basis for tumor burden, abdominal distension, cachexia and other abnormalities. These mice were sacrificed 3 days after the last administration, and then intraperitoneal tumors were excised and weighed.

To further study the therapeutic effect against ovarian cancer in this model, the survival time of those mice treated with the protocols described above was observed (five mice in each group). Mice were sacrificed when they became moribund, and the day of sacrifice of the mouse was considered as its survival time.

Histologic analysis

Intraperitoneal tumors were fixed in 4% paraformaldehyde in PBS, embedded in paraffin and cut into 3–5 μm sections. Then the sections were stained with hematoxylin and eosin (H&E). Apoptosis was evaluated by the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) technique using the DeadEnd Fluorometric TUNEL System (Promega, Madison, WI) and Hoechst 33258 following their manufacturer's protocol, respectively. For Hoechst 33258 staining, the apoptosis index was determined by analyzing the average percentage of positive cells in 5 random fields from at least three sections at a magnification of $\times 400$.

Immunohistochemical staining for NK and B cells was performed with anti-NK1.1 and anti-CD22 monoclonal antibody (1:200; BD Pharmingen, CA), respectively. Briefly, tumor sections were dewaxed, rehydrated through graded ethanol washes and incubated with 3% hydrogen peroxide for 10 min. After antigen retrieval, nonspecific binding of reagents was obtained by incubation of sections in 5% normal goat serum for 15 min. The sections were continuously incubated with anti-NK1.1 or anti-CD22 antibody at 4°C overnight, biotinylated goat anti-mouse or goat anti-rabbit secondary antibody at 37°C for 40 min and then streptavidin–biotin–horseradish peroxidase complex at 37°C for 30 min. Cellular nuclei were counterstained with ameliorative Gill's hematoxylin.

Toxicity assessment

To evaluate possible side effects in the VSVMP-p-treated mice, the animal weight was monitored every four days in the period between tumor implantation and sacrifice, and other relevant indexes such as diarrhea, anorexia, cachexia, skin ulceration or toxic death were also observed consecutively. After sacrificing, various organs (heart, liver, spleen, lung, kidney, brain, bone marrow, etc.) were harvested and fixed in 4% paraformaldehyde in PBS. These tissues were then sectioned, stained with H&E and observed by two pathologists in a blinded manner.

Statistical analysis

Comparisons of tumor weight, apoptosis index and animal weight among the different groups were performed using one-way analysis of variance (ANOVA). Survival curves were generated based on the Kaplan–Meier method and statistical significance was determined by the log-rank test. A value of $P < 0.05$ was defined as significant.

Results

VSVMP-p induced apoptosis of SKOV3 cells in vitro

In order to test the sensitivity of VSVMP-p on SKOV3 cells, the cells were seeded in 6-well plates and incubated with VSVMP-p, e-p, liposome or medium alone, respectively. Then cells were fixed, stained with Hoechst 33258. VSVMP-p induced plentiful apoptosis in contrast to control agents, which were characterized by pyknotic and fragmented nuclei (Fig. 1, top panel).

Cellular apoptosis was further verified by flow cytometric analysis using annexin V-PI double staining method (Fig. 1, bottom panel). Early apoptotic cells, represented by annexin V-positive and PI-negative cells (lower right quadrant), increased significantly in VSVMP-p-treated samples (30.8%) compared with samples treated with e-p (2.9%), liposome (0%) or medium alone (0%), respectively.

VSVMP-p inhibited intraperitoneal ovarian tumor growth in nude mice

To further study the effectiveness of VSVMP-p on inhibiting the growth of human ovarian cancer *in vivo*, we established intraperitoneal carcinomatosis in nude mice and treated them with VSVMP-p/liposome complexes and with NS, liposome alone or e-p/liposome complexes as controls. Three days after the last treatment, mice were sacrificed and intraperitoneal tumors were excised and weighed. As shown in Fig. 2, in comparison with treatments with NS, liposome alone or e-p, treatment with VSVMP-p significantly suppressed tumor growth ($p=0.006$, 0.006 , 0.010 , respectively); however, no significant difference of tumor weight was observed among control groups. Intraperitoneal administration of VSVMP-p reduced tumor weight by about $\sim 90\%$ compared with controls and one of the three VSVMP-p-treated mice exhibited no macroscopic tumors on the sacrifice day.

Increased tumor apoptosis in the VSVMP-p-treated mice

Decreased tumor burden correlated with an increased rate of tumor cell apoptosis. Immunofluorescence microscopy of TUNEL staining revealed many strongly positive nuclei in VSVMP-p-treated tumor tissues, whereas such nuclei were rare in tumor tissues of control groups (Fig. 3A, top panels). To reckon apoptosis index exactly, Hoechst 33258 staining were further performed. Similarly, it showed that VSVMP-p induced a significant enhancement of apoptosis cells (Fig. 3A, bottom panels), with the apoptosis index of $12.6 \pm 2.7\%$ compared with $2.3 \pm 0.6\%$ (NS), $3.0 \pm 0.7\%$ (liposome alone) and $2.6 \pm 0.6\%$ (e-p), respectively ($p < 0.05$, Fig. 3B).

VSVMP-p prolonged the survival of tumor-bearing mice

The beneficial effects of gene transfer of VSVMP to intraperitoneal carcinomatosis model were also reflected in the survival time. The VSVMP-p-treated group lived dramatically

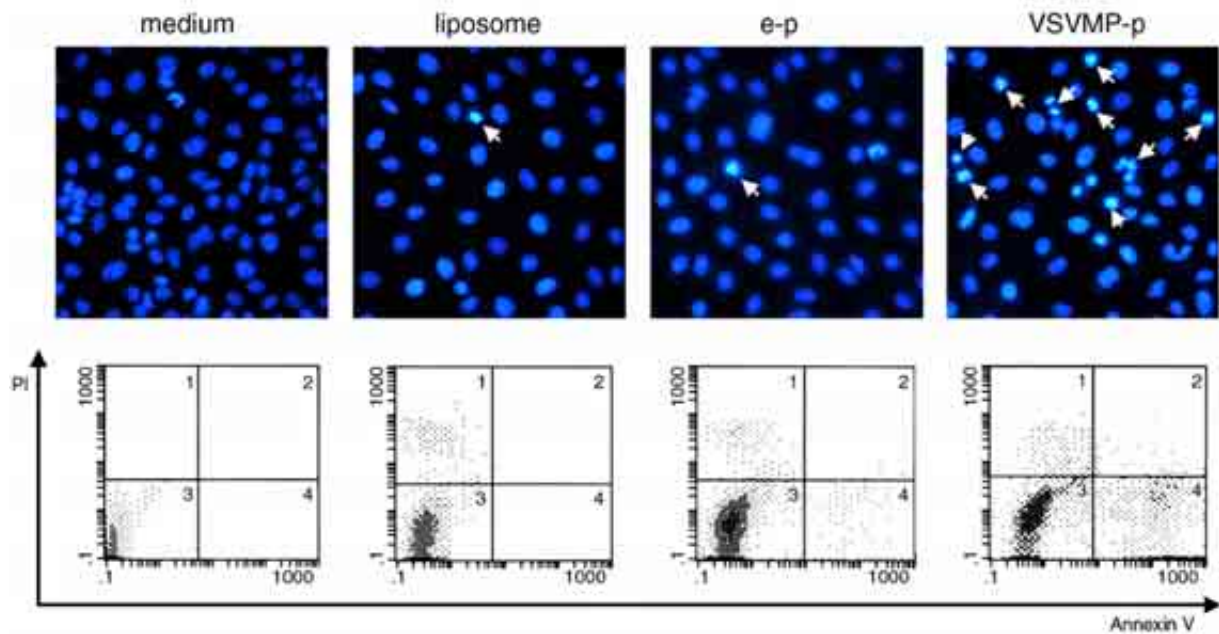


Fig. 1. VSVMP-p induced apoptosis of SKOV3 cells in vitro. Top panel: VSVMP-p resulted in plentiful apoptosis in contrast to control agents as demonstrated by Hoechst 33258 staining. Representative fields are shown and apoptotic cells are indicated by arrows. Three experiments were performed with similar results. Bottom panel: Cellular apoptosis was further verified by flow cytometric analysis using Annexin V-PI double staining method. Early apoptotic cells, represented by annexin V-positive and PI-negative cells (lower right quadrant), increased significantly in VSVMP-p-treated samples (30.8%) compared with samples treated with e-p (2.9%), liposome (0%) or medium alone (0%), respectively. The displayed result is representative of three independent experiments.

longer than the NS-, liposome-alone and e-p-treated groups ($p < 0.05$) with a median survival of 156 days in contrast to 72 days, 66 days and 79 days, respectively, as shown in the Kaplan–Meier survival curve displayed in Fig. 4. No statistically significant difference in survival among control groups was found ($p > 0.05$). Furthermore, until 200 days after i.p. inoculation when the planned experimental period ended, two VSVMP-

p-treated mice were still alive and remained healthy with no residual tumors when inspected by anatomy.

Lymphocyte accumulation of VSVMP-p-treated tumors

When examining tumor tissues stained with H&E histopathologically, we detected abundant lymphocytes around the periphery and a few even infiltrating into tumor mass of VSVMP-p-treated mice; in contrast, no obvious lymphocyte accumulation was found in the mice treated with control therapies (Figs. 5A, B). Immunohistochemical staining with anti-NK1.1 and anti-CD22 antibody showed that the lymphocyte accumulation almost consisted of NK cells; however, CD-22, a B cell-restricted transmembrane glycoprotein, was negative for these lymphocytes (Figs. 5C, D). Thus, it may be hypothesized that the antitumoral effects of VSVMP-p could be contributed not only to its ability of inducing apoptosis but also to its ability of eliciting NK cell response.

Toxicity observation

The animal weight, which was considered a surrogate for evaluation of systemic well-being, anorexia or cachexia, was monitored every four days. As shown in Fig. 6, no significant differences in weight were found among the four groups. No gross abnormalities were observed in the VSVMP-p-treated mice. Furthermore, H&E histological examination of the liver, lung, kidney, spleen, brain, heart, pancreas, intestine and bone marrow by two pathologists did not reveal any significant

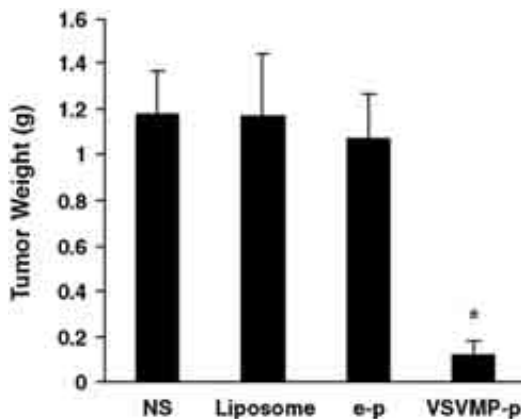


Fig. 2. VSVMP-p significantly inhibited intraperitoneal tumor growth. The intraperitoneal carcinomatosis models in nude mice were established and administered with VSVMP-p/liposome complexes, e-p/liposome complexes, liposome alone and NS, respectively. Three days after the last treatment, mice were sacrificed and intraperitoneal tumors were weighed. Values are means \pm SE; $n = 3$ mice/group. * $p < 0.05$ versus NS, liposome alone and e-p group, respectively.

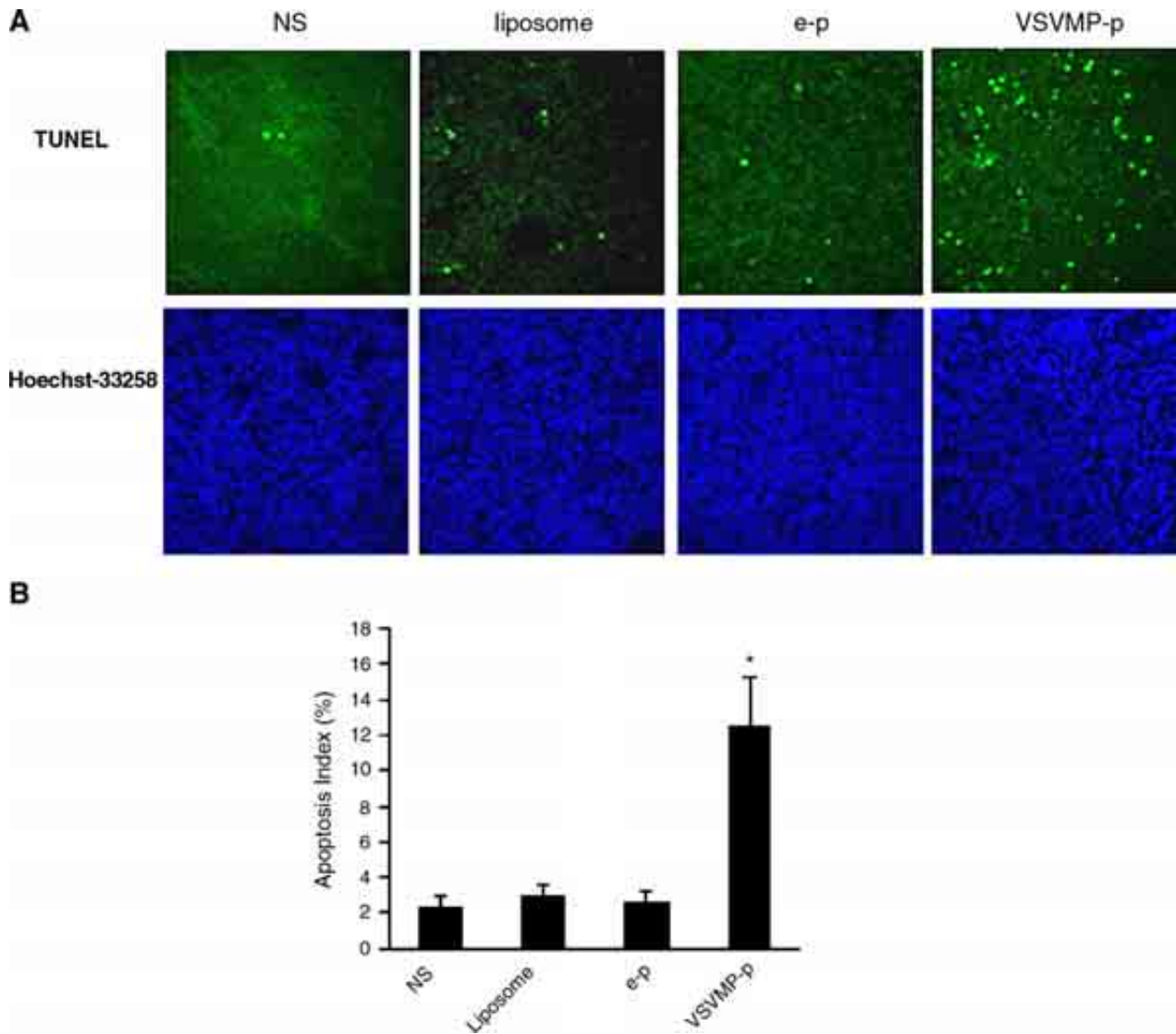


Fig. 3. Apoptosis assays of tumor tissues in different groups. (A) TUNEL and Hoechst 33258 staining of tumors revealed that VSVMP-p induced a significant enhancement of apoptotic cells in contrast to control therapies; $\times 400$. (B) apoptosis index of VSVMP-p-treated tumor was much higher than that of control groups. Values are means \pm SE. * $p < 0.05$ versus NS, liposome alone and e-p group, respectively.

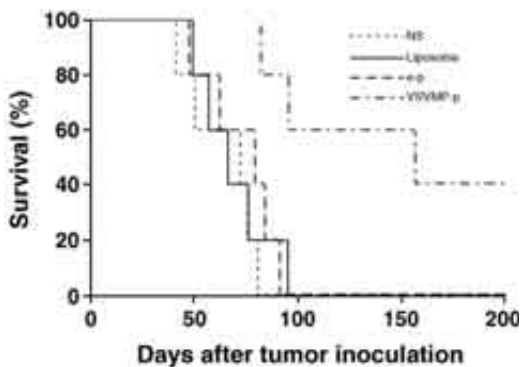


Fig. 4. Kaplan–Meier survival curve for tumor-bearing mice treated with NS, liposome alone, e-p or VSVMP-p. Survival was significantly longer in VSVMP-p-treated mice compared with control groups (log-rank test, $p < 0.05$) and there was no statistical difference among control groups ($p > 0.05$). The median survival of the VSVMP-p group was 156 days versus 72 days, 66 days and 79 days in the NS, liposome alone and e-p group, respectively.

differences among nude mice treated with either VSVMP-p or the control therapies.

Discussion

In the present study, we have demonstrated that a recombinant plasmid DNA encoding VSVMP significantly suppresses intraperitoneal xenograft growth of human ovarian cancer and prolongs survival in nude mice. This study provides proof of the principle that VSVMP possesses antitumor activity against ovarian cancer *in vivo* in the absence of other viral components, not by direct cytolysis but by induction of apoptosis and possible NK cell accumulation, and opens ways for future investigations of this approach. The reason why we choose VSVMP, a single viral component, as an antitumor approach is that it plays an important role in causing many cytopathogenesis of VSV-infected cells and that potential biohazard of VSV infection may be avoided by using MP

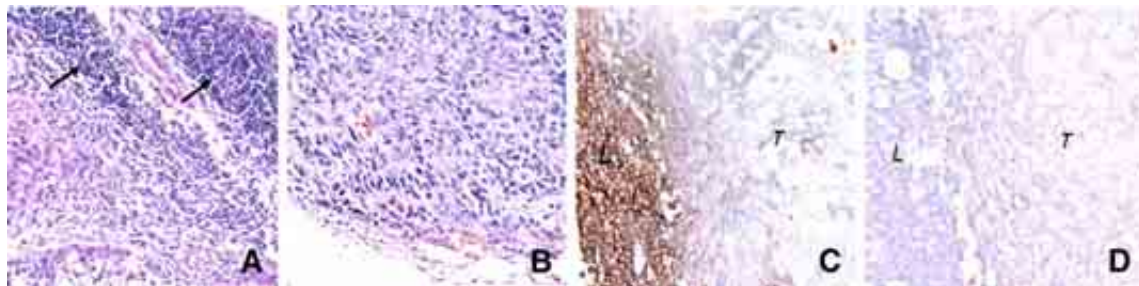


Fig. 5. H&E staining of intraperitoneal tumors three days after the last administration and immunohistochemical analysis of VSVMP-p-treated tumor. (A) Plentiful lymphocytes infiltrated the periphery of and even into tumor mass of VSVMP-p-treated mice (arrow, lymphocyte accumulation). (B) Such infiltration did not occur in the e-p-, liposome-alone- and NS-treated tumors (a photograph of e-p-treated tumor was shown). (C) Immunohistochemical staining with anti-NK1.1 antibody showed that the lymphocyte accumulation almost consisted of NK cells. (D) CD-22, a B cell-restricted transmembrane glycoprotein, was negative for these lymphocytes (T, tumor; L, lymphocytes accumulating around the tumor site). Panels A–D: $\times 400$.

alone. VSV, as well as other viruses including adenovirus, herpes simplex virus, reovirus, autonomous parvovirus, Newcastle disease virus, measles virus and many more, has been actively developed as oncolytic agents for cancer treatment [4,19,20]. It has been reported that VSV is effective in a few tumor-bearing animal models by directly killing cancer cells and stimulating the production of cytokines with antitumor activity [3–7]. However, infections of VSV in human beings may cause a mild febrile illness in some cases although most cases are asymptomatic [21], which implies that application of VSV in human beings should be cautious and limited due to its potential biohazard. Therefore, we engineered a plasmid DNA expressing VSVMP as a novel antitumor agent and tested its efficacy in an ovarian cancer model in nude mice.

To elucidate the antitumor mechanism *in vivo* of VSVMP-p, apoptosis analysis using TUNEL and Hoechst 33258 technique was performed. Our data showed that VSVMP-p resulted in abundant apoptosis of tumor cells compared with control therapies and this result was consistent with the mechanism reported previously [13,14,22]. Recent researches regarding VSVMP were predominantly focusing on its ability to induce cytopathogenesis *in vitro*, and gene transfers of VSVMP in both human ovarian tumor xenografts in nude mice (this report) and a few syngeneic tumors in the immunocompetent mice (Zhao JM et al., manuscript submitted for publication) were the first utilization of MP as an antitumor approach *in vivo*. One of the crucial therapeutic strategies against cancer is to trigger tumor-selective cell death. Apoptosis represents a universal and

efficient cellular suicide pathway and induction of tumor cell apoptosis in response to a large number of stimuli has been proved an intriguing and promising antitumor approach [23]. In this study, we employed VSVMP, reported to have the ability of inducing apoptosis, as an antitumor therapy for human ovarian cancer in nude mice and acquired satisfactory results.

In addition, an interesting and significant observation was that VSVMP-p-treated tumors were characterized by a rich recruitment of lymphocytes, almost consisting of NK cells, around and some even infiltrating into the tumor sites, which indicated the possible chemokine-like activity *in vivo* of VSVMP. Nude mice, which lack thymuses, cannot generate mature T lymphocytes and therefore are unable to mount most types of immune responses; however, they have elevated level of NK cells, which have been demonstrated to mediate potent antitumor activities [24,25] and play an important role against transplanted tumor growth in nude mice [26]. The observations of this study suggest that in addition to induction of apoptosis, another mechanism such as elicitation of NK cell accumulation may contribute to the potent antitumor efficacy of VSVMP against ovarian cancer, although further investigation is required to substantiate this suggestion. Therefore, a study to explore the activity of VSVMP to trigger antitumor immune responses will be carried out.

We used a nonviral gene delivery system by cationic liposome in this study for its minimal immunogenicity, low toxicity and feasibility of repeated administration *in vivo*. It has been proved that the pegylated liposomes, which are devoid of any specific ligand, are able to accumulate in solid tumors when administered *i.v.* as a result of a leaky microvasculature and an impaired lymphatics in tumors [27,28]; however, whether *i.p.* injection of liposome would also target tumor sites remains unclear. In our preliminary study, GFP-expressing pcDNA3.1 complexed to liposome was administered *i.p.* in peritoneal carcinomatosis model. Although no attempt was made to quantify the GFP-expression levels in tumor nodules and other normal tissues, our findings showed the GFP signal in tumors was much more evident than that in normal organs, implicating a high tumor specificity of this gene delivery system. Therefore, *i.p.* injection of VSVMP-p/liposome complexes would deliver the VSVMP gene preferentially into intraperitoneal tumor sites and induce tumor cell apoptosis without life-threatening toxicity.

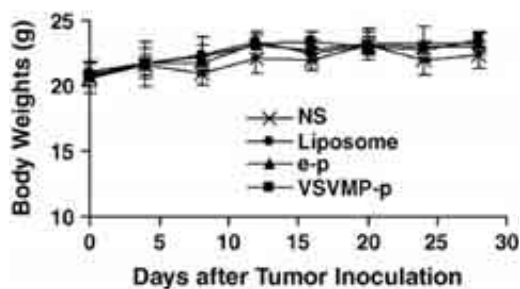


Fig. 6. Lack of toxicity-dependent weight loss in tumor-bearing mice treated with VSVMP-p. There are no significant differences in weight among the four groups ($p > 0.05$). Values are means \pm SD ($n = 8$ mice).

In conclusion, the application of VSVMP-p complexed to liposome reduced tumor burden and achieved survival improvement in peritoneal carcinomatosis models of human ovarian cancer, implying its future clinical applications in the treatment of ovarian cancer.

Acknowledgments

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