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Original Research Article

Effect of paclitaxel octreotide conjugate on human ovarian paclitaxel-resistant cell xenograft tumor model and the mechanism underlying reversal of paclitaxel resistance

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Abstract

Purpose: *To determine the effect of paclitaxel octreotide conjugate (POC) on human ovarian paclitaxel-resistant cell xenograft tumor model and the mechanism underlying reversal of paclitaxel resistance.*
Methods: *Forty female BALB/c-nu/nu mice were subcutaneously inoculated with 10^6 paclitaxel-resistant cells (a2780/taxol) per mouse during the logarithmic growth phase of ovarian cancer. They were randomly divided into four groups (control, octreotide, paclitaxel and POC). Immunohistochemical streptavidin-peroxidase (SP) method was used to determine expression of nuclear proliferation antigen (PCNA) while TUNEL method was used to assess apoptosis of human ovarian cancer metastasis. Real-time polymerase chain reaction (PCR) was used to assay mRNA expression levels of somatostatin receptor 2 (SSTR2), multidrug-resistant gene (MDR1), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and acetylated tubulin (α-tubulin and β-III-tubulin), while the corresponding protein expressions were assayed using western blotting.*
Results: *Immunohistochemical SP showed significantly lower PCNA levels in octreotide, paclitaxel and POC groups than in control mice, but that of POC mice was significantly reduced, relative to those of octreotide and paclitaxel groups ($p < 0.05$). There were significantly higher expression levels of SSTR2 mRNA and protein in octreotide, paclitaxel and POC groups than in control mice, but they were significantly higher in POC group than in octreotide and paclitaxel groups ($p < 0.05$). The mRNA and protein expressions of other factors in POC mice were significantly lower than those in both octreotide and paclitaxel groups ($p < 0.05$).*
Conclusion: *Paclitaxel-octreotide conjugate effectively inhibits the growth of a2780/taxol xenografts in nude mice, induces tumor cell apoptosis, and suppresses tumor cell growth via mechanism involving enhancement of SSTR2 expression, and decreases in levels of acetylated tubulin, matrix metalloproteinase-9, and vascular endothelial growth factor.*

Keywords: *Paclitaxel octreotide conjugate, Ovarian cancer, Xenograft tumor model, Nude mice, Reversal, Paclitaxel resistant cells, Mechanism*

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INTRODUCTION

Ovarian cancer refers to malignant tumors in the ovaries, and it is currently among the 3 main malignancies in women. As symptoms do not show at the early stage of ovary carcinoma, early diagnosis is relatively difficult. Even if there are symptoms, they are not specific. In addition, due to limited screening techniques, most patients are diagnosed with advanced disease. Thus, for most subjects, diagnosis of the disease occurs late, such that its mortality rate is the highest among gynecological malignant tumors, and it is the most severe disease that seriously threatens women's health [1-2]. At present, platinum and paclitaxel combination is the main pancreatic chemotherapeutic regimen for postoperative treatment of ovarian cancer, but long-term application makes the tumor cells acquire drug resistance, resulting in poor efficacy of subsequent chemotherapy [3]. Therefore, the development of novel anti-tumor medications is crucial for the treatment of ovarian cancer which ultimately will lead to an improvement in patients' quality of life [4]. In recent years, targeted therapy of tumors has received extensive attention from scholars in the medical community. Natural somatostatin (SST) is a cyclic polypeptide that inhibits the secretion of a variety of hormones. In addition, it inhibits cell proliferation and promotes cell apoptosis. The physiological functions of SST occur through the SSTR of target cells [5-7]. Octreotide is the most representative somatostatin analogue (SSTA) at present, and it produces good therapeutic effects on neuroendocrine tumors, gastrointestinal bleeding, malignant tumor ascites and other aspects [8]. Previous studies have found that paclitaxel octreotide conjugate (POC) has high targeting ability. Whether in *in vivo* or nude mice bearing tumors, paclitaxel octreotide conjugate suppresses tumor cell growth and increases cancer cell sensitivity to paclitaxel [9]. In the present work, the influence of POC on human ovary carcinoma cells insensitive to paclitaxel (a2780/taxol) was investigated in nude mouse xenograft tumor model, and the associated mechanism linked to reversal of paclitaxel resistance was also studied.

EXPERIMENTAL

Cell line

Cell line (a2780/taxol) was purchased from Shanghai QiaoDu Biotech. Co. Ltd, China.

Animals

Female BALB/c-nu/nu nude mice were obtained from Beijing Weitong Lihua Lab. Animal Technol. Co. Ltd, China. The average body weight of mice was 27.5 ± 2.5 g, and the average age was about 6 ± 1 weeks. The mice were housed at room temperature of 22 - 24 ℃, relative humidity of 40 – 60 %, and noise ≤ 45 dB, and were given free access to conventional feed (Laboratory rat maintenance feed, Jiangsu Xietong Medical Bioengineering Co., Ltd.) and water.

Drugs and reagents

Paclitaxel octreotide conjugate (POC) was provided by Xi'an Ruixi Biotechnology Co. Ltd, China. Phosphate-buffered saline (PBS) was obtained from Shanghai Kemin Biotechnology Co. Ltd. The other drugs and reagents, and their suppliers (in brackets) were: fetal bovine serum (Wuhan Punuosai Life Technology Co., Ltd); 0.25 % trypsin (Amej Technology Co. Ltd), dimethyl sulphoxide (DMSO) (Beijing Xinshengyuan Biomedical Technology Co. Ltd); RPMI 1640 (Hangzhou Jinuo Biomedical Technology Co. Ltd); paclitaxel (Shanghai Baoman Biotechnology Co. Ltd); isoflurane (Shanghai Yuyan Scientific Instrument Co. Ltd); octreotide (Shanghai Jingke Chemical Technol. Co. Ltd); hematoxylin and eosin dye solutions (Asbixin (Shanghai) Biotechnology Co. Ltd); neutral gum (Hong Kong Gisi Enbei International Trading Co. Ltd); bicinchoninic acid (BCA) protein quantitative detection kit (Shenyang Wanlei Biotechnology Co. Ltd); radioimmunoprecipitation assay (RIPA) lysing buffer (Harbin Xinhai Genetic Testing Co. Ltd), and phenylmethanesulfonyl fluoride (PMSF) (Shanghai Hengfei Biotechnology Co. Ltd).

Instruments

The instruments used, and the sources were carbon dioxide (CO_2) incubator (Shanghai Fuze Trading Co. Ltd); super clean workbench (Escor Trading Co., Ltd); inverted phase contrast microscope (Nanjing Beideng Medical Co. Ltd); imager *in vivo* (Suzhou Newman Analytical Instrument Co. Ltd); embedding machine (Beijing Jiayuan Xingye Technology Co. Ltd); pathological microtome (Beijing Jiayuan Xingye Technology Co. Ltd); upright light microscope (Puma Precision Medical Technology (Beijing) Co. Ltd); electric thermostatic water bath box (Nanjing Beideng Medical Co. Ltd); constant temperature magnetic mixer (Shanghai Fuze Trading Co. Ltd); vertical electrophoresis apparatus (Hangzhou Notting Scientific

Equipment Co. Ltd), and electric transfer tank (Guangzhou Jianlun Biotechnology Co. Ltd).

Cell resuscitation and cryopreservation

A suspension of the a2780/taxol cells was centrifuged at 1000 rpm (revolution per minute) for 5 min. After discarding the supernatant, 6 mL of complete medium (10 % fetal bovine serum + 1 % penicillin-streptomycin + 89 % RMPI 1640 medium) was added, and the cell suspension was aspirated into a cell culture bottle which was incubated at 37 ℃ in 5 % carbon dioxide medium [10]. The medium was refreshed on the next day, and the cell culture was rinsed in 2 - 3 mL of PBS, followed by digestion using 1 - 2 mL of 0.25 % trypsin. The digestion was terminated by addition of 4 mL of complete medium. The cell suspension was then centrifuged at 1000 rpm for 5 min, after which the cells were aspirated into a cell culture flask in a medium containing paclitaxel at a concentration of 800 ng/mL and incubated at 37 ℃ in a 5 % CO_2 incubator. On the next day, the medium was changed, and the cells were rinsed in PBS and re-digested with trypsin as before. After digestion, the cells were again centrifuged at 1000 rpm for 5 min, and the free cells were taken up in 1.5 mL of cell cryopreservation solution in a cell cryopreservation tube at 4 ℃ for 30 min, -20 ℃ for 1 h, and -80 ℃ overnight [10]. Thereafter, the cells were transferred into liquid nitrogen for long-term storage.

Establishment of animal model of paclitaxel-resistant ovarian cancer cells

The a2780/taxol in their logarithmic growth phase were made into single-cell suspension. A total of 50 female nude mice were given subcutaneous inoculation on the right peddler's nest close to the posterior, at a dose of 10^6 cells per mouse [11]. During the observation period of 4 weeks, the feeding and activity of the nude mice were monitored every day, and the growth of transplanted tumors was recorded. After the cancer grew to a diameter of about 1 cm, 40 female nude mice were randomly divided into four groups, with 10 mice in each group. The four groups were control group (injected with 0.1 mL of saline on days 1, 8 and 15 post-cancer formation); octreotide group (injected with 0.1 mL of 150 nmol/kg octreotide on days 1, 8 and 15 post-cancer formation); paclitaxel group (injected with 0.1 mL of 150 nmol/kg paclitaxel on days 1, 8 and 15 post-cancer formation), and POC group (injected with 0.1 mL of 150 nmol/kg POC on similar days as before, post-cancer formation). All injections were given via the tail vein. The study received approval (no. HMUA2022011) from the Animal Ethics Authority of The Fourth Hospital, Hebei Medical University, Shijiazhuang, China and was conducted in line with NIH guidelines [12].

Evaluation of parameters

The growth of transplanted tumors in nude mice in each group was monitored, as well as the general state of nude mice every day. The short (D) and long diameters (L) of tumors were measured using Vernier calipers [13]. The weight of each nude mouse was also recorded, and the tumor volume (Tv) was calculated according to Eq 1 [14].

$$Tv = (L \times D^2)/2 \quad (1)$$

On the 15th day after drug injection, the nude mice were euthanized (cervical dislocation), transplanted tumors were stripped, the volumes of transplanted tumors were measured, and the tumor inhibition rate was calculated as shown in Eq 2.

$$\text{Tumor inhibition (\%)} = \{(Tv_{(blank)} - Tv_{(study)})/Tv(\text{blank})\}100 \quad (2)$$

where Tv = average tumor volume.

Hematoxylin & eosin (H&E) staining

Fresh tissue was fixed in 4 % formalin for more than 48 h and subjected to routine dehydration, embedding, and sectioning [15]. Sections were passed through xylene Ⅰ (20 min), xylene Ⅱ for another 20 min, water-free alcohol (10 min), water-free hexanol Ⅱ (10 min), 95 % ethanol (for 5 min), 90 % ethanol (another 5 min), and in 80 and 70 % alcohol (5 min each), in that order, after which they were rinsed in distilled H_2O. This was followed by staining with Harris hematoxylin (for nuclei) for 3 - 8 min, and differentiation using 1 % alcoholic hydrochloric acid, blue-black coloring using 0.6 % NH_3, and rinsing in H_2O. Then, counter-staining with eosin (for cytoplasm) was done, and the stained samples were dehydrated, sealed, examined microscopically, and analyzed using image acquisition [15].

TdT-mediated dUTP nick end labeling (TUNEL) assay

Cervical dislocation was used for sacrificing mice in all groups, and tumor tissues were isolated and snap-frozen [16]. Three tumor tissue specimens were randomly taken from each group, embedded in optimal cutting temperature compound (OCT) mixture

(embedding agent), frozen, and sliced into 4-μm frozen sections, followed by apoptosis assay using TUNEL assay kits in line with the instructions of the manufacturers. The apoptosis in the sections of the four groups was examined under the light microscope, and the apoptotic cells were identified by the presence of brownish yellow particles in the cells or brownish yellow color of the whole cell [16].

Immunohistochemical streptavidin-peroxidase (SP) method

The transplanted tumor tissues of nude mice were fixed and preserved with 4 % paraformaldehyde [17]. After the morphological characteristics of tumor cells were examined using conventional H & E staining, the expression of nuclear-proliferating antigen (PCNA) in subcutaneous xenografts of nude mice was determined using immunohistochemical SP method. The tumor tissues were subjected to conventional paraffin embedding, sectioning, dewaxing and hydration, followed by addition of rabbit anti-human PCNA monoclonal antibody working solution and overnight incubation at 4 ℃. Then, the slices were PBS-rinsed 3 times, followed by incubation with horseradish peroxidase-conjugated 2° immunoglobulin working solution at laboratory temperature for 30 min. The slices were rinsed thrice with PBS, and subjected to 3,3'-diaminobenzidine (DAB) color development, followed by hematoxylin comparative staining.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Complete RNA extraction was done with TRIzol reagent, and the RNA was reverse-transcribed to cDNA, followed by PCR amplification. Relative mRNA expressions were calculated using $2^{-\Delta\Delta\Delta Ct}$ method [18].

Immunoblot assay

Total cellular and tissue protein extractions were done using RIPA reagent. Proteins in samples were quantified using the bicinchoninic acid assay (BCA) method [19]. Then, the proteins were subjected to SDS-PAGE and transferred to polyvinylidene difluoride filaments (PVDF) which were thereafter blocked with 5 % fat-free milk solution. Then, the membranes were incubated overnight at 4 °C with relevant primary antibodies, followed by incubation with horse radish peroxidase-conjugated secondary antibody at room temperature for 2 h. The bands were subjected to enhanced chemiluminescence, and relative protein expression levels were calculated using gel image analysis [19].

Statistics

The data analysis was done using SPSS 20.0 software. All measurement data are expressed as mean ± standard deviation (SD), and *t*-test was used for comparison between groups. Count data are expressed as percentages, and comparisons between groups were done with χ^2 test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Tumor volume and mass of human ovarian metastatic tumor

The tumor volumes were significantly lower in the octreotide, paclitaxel and POC groups than in control mice, but tumor volume was significantly lower in POC-treated mice than in both octreotide and paclitaxel groups ($p < 0.05$). The body weight of the POC group was significantly higher than those of the octreotide and paclitaxel groups ($p < 0.05$; Table 1).

Table 1: Tissue volume and body mass of human ovarian cancer metastatic tumor in nude mice of each group

Group	Tumor volume (mm^3)	Body mass (g)
Control	805.0±15.38	-
Octreotide	328.79±10.85^a	60.95±3.75
Paclitaxel	214.35±6.95^{a,b}	73.88±4.51^b
POC	90.71±5.39^{a,b,c}	89.56±3.48^{b,c}

$^a P < 0.05$, vs. control; $^b p < 0.05$, vs. octreotide group; $^c p < 0.05$, vs. paclitaxel and octreotide groups. Values are mean ± SD

Table 2: Apoptosis of human ovarian cancer metastatic tumor tissues in nude mice of each group

Group	Apoptosis (%)
Control	8.68±1.26
Octreotide	18.93±2.02^a
Paclitaxel	19.68±1.83^a
POC	34.83±3.74^{a,b}

$^a P < 0.05$, compared with control group; $^b p < 0.05$, compared with octreotide and paclitaxel groups; Values are mean ± SD

Apoptosis of human ovarian cancer metastatic tumor tissues

Results from TUNEL showed significantly higher ($p < 0.05$) percentage apoptosis of tumor tissues in all groups, when compared to control mice,

with POC group showing the most significant ($p < 0.05$) degree of apoptosis in comparison with the octreotide and paclitaxel groups. These results are shown in Table 2.

Histo-morphological characteristics of A2780/taxol cell metastasis

The octreotide, paclitaxel and POC groups had different degrees of necrotic lesions, inflammation-associated cell infiltrates, and fibrotic lesions, as well as small amounts of nuclear pyknosis and lysis, relative to control mice *(results not shown)*, but the effect on POC mice was significantly better than those on the other two groups ($p < 0.05$).

Table 3: PCNA expression in human ovarian cancer metastasis tissues of nude mice in each group

Group	PCNA expression
Control	2.06 ± 0.23
Octreotide	1.19 ± 0.15[a]
Paclitaxel	1.32 ± 0.17[a]
POC	0.64 ± 0.07[a,b]

[a]$P < 0.05$, compared with the control group; [b]$p < 0.05$, compared with the octreotide and paclitaxel groups; Values are presented as mean ± SD

Expression level of PCNA in human ovarian cancer metastasis tissue

Immunohistochemical SP showed significantly lower expression levels of PCNA in cancer tissues in all the groups than in control mice, and lower PCNA levels were seen in the POC group than in octreotide and paclitaxel groups ($p < 0.05$; Table 3).

mRNA expressions of associated factors

There were significantly higher mRNA expression levels of somatostatin receptor 2 (SSTR2) in tumor tissues of octreotide, paclitaxel and POC groups compared to the control group, but it was significantly higher in the POC group than in the octreotide and paclitaxel groups ($p < 0.05$). The tissue mRNA expressions of the multidrug-resistant gene (MDR1), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and acetylated tubulin in all the groups were significantly lower than those of the control, with the POC group showing the most significant effects when compared with the paclitaxel and octreotide groups ($p < 0.05$; Table 4).

Protein levels of associated factors

There were significant upregulations of SSTR2 protein in octreotide, paclitaxel and POC groups, relative to control mice ($p < 0.05$). Interestingly the protein expression level of SSTR2 was noted to be significantly higher in the POC group than in both the octreotide group and paclitaxel groups ($p < 0.05$). In contrast, protein levels of MMP-9, α-tubulin, β-Ⅲ tubulin, VEGF and MDR1 were significantly reduced in POC group, relative to octreotide and paclitaxel groups (Table 5).

DISCUSSION

The combined use of platinum and paclitaxel is the preferred chemotherapy for ovarian cancer at present [20]. However, it is associated with serious drug resistance which leads to relapse in some patients, and hence treatment failure.

Table 4: SSTR2, MDR1, VEGF, MMP-9 α-tubulin β; comparison of Ⅲ - tubulin mRNA expression

Group	SSTR2	MDR1	α-Tubulin	β Ⅲ-tubulin	VEGF	MMP-9
Control	1.26±0.08	0.89±0.11	1.02±0.25	1.02±0.21	1.05±0.31	0.96±0.21
Octreotide	1.97±0.15[a]	0.41±0.18[a]	0.63±0.21[a]	0.82±0.23[a]	0.89±0.34[a]	0.86±0.25[a]
Paclitaxel	3.85±0.33[a,b]	0.53±0.26[a,b]	0.51±0.14[a,b]	0.69±0.17[a,b]	0.68±0.28[a,b]	0.61±0.19[a,b]
POC	8.07±0.86[a,b,c]	0.09±0.02[a,b,c]	0.11±0.06[a,b,c]	0.26±0.04 [a,b,c]	0.18±0.03 [a,b,c]	0.21±0.08 [a,b,c]

[a]$P < 0.05$, vs. control; [b]$p < 0.05$, vs. octreotide-treated mice; [c]$p < 0.05$, vs. paclitaxel-treated mice. Values are mean ± SD

Table 5: Comparison of protein levels of factors among the groups

Group	SSTR2	MDR1	α-Tubulin	βⅢ–tubulin	VEGF	MMP-9
Control	1.20±0.16	1.19±0.24	1.12±0.35	1.15±0.25	1.12±0.23	0.97±0.22
Octreotide	1.52±0.31[a]	0.78±0.19[a]	0.93±0.31[a]	0.98±0.22[a]	0.81±0.18[a]	0.75±0.16[a]
Paclitaxel	1.89±0.29[a,b]	0.89±0.24[a,b]	0.85±0.21[a,b]	0.76±0.17[ab]	0.95±0.12[a,b]	0.69±0.12[a,b]
POC	3.18±0.41[a,b,c]	0.31±0.08[a,b,c]	0.65±0.16[a,b,c]	0.34±0.05[a,b,c]	0.54±0.06[a,b,c]	0.21±0.04[a,b,c]

[a]$P < 0.05$, compared with control group; [b]$p < 0.05$, compared with octreotide group; [c]$p < 0.05$, compared with paclitaxel group. Values are mean ± SD

Molecular boot therapy, a typical representative of which is SSTR, has become the focus of interest due to its advantages of enhanced drug effectiveness and low toxic side effects [21]. Presently, octreotide a somatostatin analogue is widely used in clinics and has a long half-life and strong effect. It inhibits neuroendocrine tumors, gastrointestinal tumors, breast cancer, and leukemia. By combining with the SSTR of the target cells, it produces low adverse response during clinical use [22]. Octreotide inhibits the proliferation of SSTR2-expressing cells. Most tumor tissues express SSTR2. Therefore, some studies have coupled somatostatin analogues with doxorubicin anticancer compounds and found that these complexes produced whiplash and toxic effects on cancer cells expressing SSTR, while the toxicity to normal tissues was low. It has been reported that coupling SST analogues with paclitaxel enhanced the killing effect of paclitaxel on breast cancer cells and their stem cells [23]. The protein PCNA is present only in normal proliferating and tumor cells. Recent studies have found that PCNA is closely related to cellular DNA synthesis; it regulates cell proliferation, and it is a good indicator of cell proliferation [24].

In this study, the tumor volumes of all the groups were significantly lower than those of the control group, and the tumor volume of the POC group was lower than those of the octreotide and paclitaxel groups. Mice in POC had significantly higher weights than mice in octreotide and paclitaxel groups. This indicates that the tumor tissue in the POC group was significantly inhibited and the growth rate was significantly slowed down. TUNEL assay showed a higher percentage apoptosis of tumor tissues in octreotide, paclitaxel and POC groups than in control mice, and percentage apoptosis was higher in POC group than in octreotide and paclitaxel groups. This suggests that compared to a single paclitaxel or octreotide, POC significantly promoted apoptosis of tumor tissue cells. The immunohistochemical SP method showed that the expressions of PCNA in tumor tissues of all the groups were significantly lower than that of the control group, while that of the POC group was lower than those of the octreotide and paclitaxel groups. Tumor cells have strong proliferative activity, and PCNA can be used as an indicator to evaluate cell proliferation status, indicating that POC inhibits tumor proliferation. There were varying degrees of cancer necrotic changes, infiltrates of inflammatory cells, and fibrotic lesions in octreotide-treated mice, paclitaxel-treated mice, and conjugate group, as well as low levels of nuclear pyknosis and lysis, with the impact in POC mice being the best. Summarizing the above results, it may be concluded that, compared to a single intervention with paclitaxel or octreotide, POC more effectively inhibited tumor tissue proliferation and induced tumor cell apoptosis, thereby reversing tumor drug resistance. Thus, it has practical clinical value.

Studies have shown that SSTA reverses drug insensitivity through suppression of MDR1 expression regulated by pi3k/Akt signaling pathway in combination with SSTR1a and SSTR2 [25]. It is known that VEGF is an important growth factor that enhances angiogenesis and the proliferation and migration of vascular endothelial cells, and significantly regulates the permeability of blood vessels. The MMPs increase the invasiveness of tumor cells by degrading the extracellular matrix. Thus, high expression levels of MMP-9 are particularly related to malignant ovarian cancer. Research has shown that down-regulation of β-Ⅲ-tubulin protein with surface method increased the sensitivity of ovarian cancer drug-resistant cell lines to paclitaxel, while its overexpression resulted in paclitaxel chemoresistance [26]. The expression of α-tubulin is increased in drug-resistant cell lines of breast cancer. Reducing its expression level increased the susceptibility of drug-insensitive cells to chemotherapy. It has been established that MDR1 is a multidrug resistance gene, and its overexpression reduces intracellular drug concentration, leading to drug resistance of tumor cells. In this study, the mRNA and protein expressions of SSTR2 were significantly higher in the octreotide, paclitaxel and POC groups than in the control group, and they were significantly higher in POC group than in the octreotide and paclitaxel groups. The mRNA and protein expressions of MMP-9, α- and β-Ⅲ tubulins, VEGF, and MDR1 in the POC mice were significantly lower than the corresponding expressions in the octreotide and paclitaxel groups. These results suggest that the anticancer mechanism of POC is associated with suppression of MMP-1, MDR1, α- and β-Ⅲ-tubulin and VEGF, and enhancement of SSTR2 expression.

CONCLUSION

This study has demonstrated that POC effectively inhibits the growth of a2780/taxol xenografts in nude mice, induces tumor cell apoptosis, and inhibits tumor cell proliferation. Its anticancer mechanism is likely due to suppression of MMP-1, MDR1, α- and β-Ⅲ-tubulin and VEGF, and enhancement of SSTR2 expression.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities about claims relating to the content of this article will be borne by the authors. Ying Wang designed the study, supervised the data collection, and analyzed the data. Hui Guo interpreted the data and prepared the manuscript for publication. Jing Ma and Shifa Yuan supervised the data collection, analyzed the data, and reviewed the draft of the manuscript.

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REFERENCES

1. *Jiang SY, Chang H, Fan DY, Deng SJ. The roles of icariin on the proliferation and apoptosis abilities of human oophoroma cells and multi-drug resistant cell line. Sichuan Da Xue Xue Bao Yi Xue Ban 2018; 49(4): 530-534.*
2. *Dos Santos NG, Stephan LR, Otero A, Iglesias C, Castilho-Noll MSM. How do free-floating macrophytes influence interactions between planktivorous fish and zooplankton in tropical environments? An in-lake mesocosm approach. Hydrobiologia 2020; 847, 1357-1370.*
3. *Gonçalves IS, Sampaio J, Félix J, Silva AC, Fornelos G, Silva PT. Oophoropexy to the Round Ligament after Recurrent Adnexal Torsion. Rev Bras Ginecol Obstet 2018; 40(11): 726-730.*
4. *Arduino I, Liu Z, Iacobazzi RM, Lopedota AA, Lopalco A, Cutrignelli A, Laquintana V, Porcelli L, Azzariti A, Franco M, et al. Microfluidic preparation and in vitro evaluation of iRGD-functionalized solid lipid nanoparticles for targeted delivery of paclitaxel to tumor cells. Int J Pharm 2021; 610: 121246.*
5. *Li L, Yang M, Li R, Hu J, Yu L, Qian X. iRGD Co-Administration with paclitaxel-loaded PLGA nanoparticles enhance targeting and antitumor effect in colorectal cancer treatment. Anticancer Agents Med Chem 2021; 21(7): 910-918.*
6. *Gao Y, Chen J, Zhang X, Xie H, Wang Y, Guo S. Quantification of paclitaxel and polyaspartate paclitaxel conjugate in beagle plasma: application to a pharmacokinetic study. J Chromatogr Sci 2017; 55(3): 222-231.*
7. *Simón-Gracia L, Scodeller P, Fisher WS, Sidorenko V, Steffes VM, Ewert KK, Safinya CR, Teesalu T. Paclitaxel-loaded cationic fluid lipid nanodiscs and liposomes with brush-conformation PEG chains penetrate breast tumors and trigger caspase-3 activation. ACS Appl Mater Interfaces 2022; 14(51): 56613-56622.*
8. *Yan Q, Yang Y, Chen W, Hu J, Yang D. Construction of polymer-paclitaxel conjugate linked via a disulfide bond. Mater Sci Eng C Mater Biol Appl 2016; 58: 580-585.*
9. *Liu Y, Xia H, Wang Y, Han W, Qin J, Gao W, Qu X, Wang X. Targeted paclitaxel-octreotide conjugates inhibited the growth of paclitaxel-resistant human non-small cell lung cancer A549 cells in vitro. Thorac Cancer 2021; 12(22): 3053-3061.*
10. *Jing W, Tuxiu X, Xiaobing L, Guijun J, Lulu K, Jie J, Lu Y, Liying Z, Xiaoxing X, Jingjun L. LncRNA GAS5/miR-137 is a hypoxia-responsive axis involved in cardiac arrest and cardiopulmonary cerebral resuscitation. Front Immunol 2022; 12: 790750.*
11. *Fan LL, Chen X, Zhang XY, Li ZM, Fan XM, Shen Y. Octreotide-paclitaxel conjugate reverses paclitaxel resistance by p38 mitogen-activated protein kinase (MAPK) signaling pathway in A2780/taxol human ovarian cancer cells. Med Sci Monit 2020; 26: e922612.*
12. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*
13. *Deng X, Li X, Guo X, Lu Y, Xie Y, Huang X, Lin J, Tan W, Wang C. Myeloid-derived suppressor cells promote tumor growth and sorafenib resistance by inducing FGF1 upregulation and fibrosis. Neoplasia 2022; 28: 100788.*

14. *Qian X, Wang Y, Xu Y, Ma L, Xue N, Jiang Z, Cao Y, Akakuru OU, Li J, Zhang S, Wu A. Active targeting nano-scale bubbles enhanced ultrasound cavitation chemotherapy in Y receptor-overexpressed breast cancer. J Mater Chem B 2020; 8(31): 6837-6844.*
15. *Jiang L, Yang M, He S, Li Z, Li H, Niu T, Xie D, Mei Y, He X, Wei L, et al. MMP12 knockout prevents weight and muscle loss in tumor-bearing mice. BMC Cancer. 2021; 21(1): 1297.*
16. *Hou J, Lei Z, Cui L, Hou Y, Yang L, An R, Wang Q, Li S, Zhang H, Zhang L. Polystyrene microplastics lead to pyroptosis and apoptosis of ovarian granulosa cells via NLRP3/Caspase-1 signaling pathway in rats. Ecotoxicol Environ Saf 2021; 212: 112012.*
17. *Yang J, Jing L, Liu CJ, Bai WW, Zhu SC. 53BP1 regulates cell cycle arrest in esophageal cancer model. Eur Rev Med Pharmacol Sci 2019; 23(2): 604-612.*
18. *Rossant J, Nagy A. Preparation of polymerase chain reaction templates from embryonic and adult mouse tissue samples. Cold Spring Harb Protoc 2019; 2019(3).*
19. *Petrozziello T, Mills AN, Farhan SMK, Mueller KA, Granucci EJ, Glajch KE, Chan J, Chew S, Berry JD, Sadri-Vakili G. Lipocalin-2 is increased in amyotrophic lateral sclerosis. Muscle Nerve 2020; 62(2): 272-283.*
20. *Wang L, Li S, Zhu D, Qin Y, Wang X, Hong Z, Han Z. Effectiveness and safety of nab-paclitaxel and platinum as first-line chemotherapy for ovarian cancer: a retrospective study. J Gynecol Oncol 2023; 34(4): e44.*
21. *Denkert C, Seither F, Schneeweiss A, Link T, Blohmer JU, Just M, Wimberger P, Forberger A, Tesch H, Jackisch C, et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. Lancet Oncol 2021; 22(8): 1151-1161.*
22. *Raposo MDA, Pina A, Dal Corso A, Arosio D, Belvisi L, Pignataro L, Caruso M, Gennari C. Multivalency Increases the Binding Strength of RGD Peptidomimetic-Paclitaxel Conjugates to Integrin αV β3. Chem 2017; 23(58): 14410-14415.*
23. *Rostovtseva TK, Gurnev PA, Hoogerheide DP, Rovini A, Sirajuddin M, Bezrukov SM. Sequence diversity of tubulin isotypes in regulation of the mitochondrial voltage-dependent anion channel. J Biol Chem 2018; 293(28): 10949-10962.*
24. *Zhang CX, Wu CT, Xiao L, Tang SH. The diagnostic and clinicopathological value of trefoil factor 3 in patients with gastric cancer: a systematic review and meta-analysis. Biomarkers 2021; 26(2): 95-102.*
25. *Tian R, Jacobson O, Niu G, Kiesewetter DO, Wang Z, Zhu G, Ma Y, Liu G, Chen X. Evans blue attachment enhances somatostatin receptor subtype-2 imaging and radiotherapy. Theranostics 2018; 8(3): 735-745.*
26. *Vo NT, Bols NC. Demonstration of primary cilia and acetylated α-tubulin in fish endothelial, epithelial and fibroblast cell lines. Fish Physiol Biochem 2016; 42(1): 29-38.*