Pharmacodynamic study of ^{131}I-labeled CA215 antibody on an animal model of estrogen-resistant OC-3-VGH ovarian cancer

XIANG-YUN LIU[1], XIN SU[2], CHEN-JING XIE[2], LEI LI[2], JIAN-YAN YAN[2] and ZU-YUE SUN[2]

[1]Key Laboratory of Exercise and Health Sciences, Shanghai University of Sport, Ministry of Education, Shanghai 200438; [2]Shanghai Institute of Planned Parenthood Research, Shanghai 200032, P.R. China

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Abstract. The aim of the present study was to explore the inhibitory effect of ^{131}I-labeled ovarian cancer antigen 215 (^{131}I-CA215) antibody on human OC-3-VGH ovarian cancer. A subcutaneous transplanted tumor model of estrogen-resistant human OC-3-VGH ovarian cancer in nude mice was established. The model mice were randomly divided into seven groups, which were the negative control (NC), positive control (PC; 60 mg/kg cyclophosphamide), high-dose CA215 antibody (HA; 10 mg/kg), low-dose CA215 antibody (LA; 2 mg/kg), high-dose ^{131}I-CA215 antibody (^{131}I-HA; 10 mg/kg + 125 μCi), medium-dose ^{131}I-CA215 antibody (^{131}I-MA; 6 mg/kg + 75 μCi) and low-dose ^{131}I-CA215 antibody (^{131}I-LA; 2 mg/kg + 25 μCi) groups. Each group received intraperitoneal administration for 14 consecutive days. At 24 h after the final administration, the tumor was removed and weighed to calculate the tumor inhibition rate (TIR) and the relative tumor increase rate (T/C). Compared with the NC group, the HA group, as well as the ^{31}I-HA and ^{131}I-MA antibody groups, exhibited significantly inhibited tumor growth. The relative T/C values were 54, 30 and 48%, respectively, and the TIRs were 33.59, 64.89 and 45.80%, respectively. All differences were statistically significant. The difference between the HA and ^{131}I-HA groups also presented statistical significance. CA215 and ^{131}I-CA215 antibodies can markedly inhibit OC-3-VGH ovarian cancer. The high-dose ^{131}I-CA215 antibody demonstrated a clear synergetic effect.

Correspondence to: Dr Xiang-Yun Liu, Key Laboratory of Exercise and Health Sciences, Room 417, Research Activities Building, Shanghai University of Sport, Ministry of Education, 188 Hengren Road, Shanghai 200438, P.R. China
E-mail: xyunliu656@163.com

Dr Zu-Yue Sun, Shanghai Institute of Planned Parenthood Research, 2140 Xietu Road, Shanghai 200032, P.R. China
E-mail: szyue_321@163.com

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Introduction

Ovarian cancer seriously impedes the reproductive health of females. Data from the American Cancer Society show that ovarian cancer is the ninth most common type of cancer in females (excluding skin cancer). It ranks fifth as the cause of cancer mortality in females. In 2013, it was estimated that there were ~22,240 new cases of ovarian cancer and ~14,230 individuals succumbed to this cancer (1). In China, ovarian cancer is the third most frequent cause of mortality among females (2). However, due to factors including environmental factors and drug treatment, the cell types and subtypes of ovarian cancer are increasing. For example, the OC-3-VGH cell line is a line of ovarian cancer that seldom expresses the estrogen receptor (3), and is insensitive to estrogen. The OC-3-VGH cell line was first successfully isolated in Taiwan (3). However, few studies have been conducted on this tumor type. The successful establishment of an animal model of OC-3-VGH would provide a drug study platform for the treatment of patients with estrogen-resistant ovarian cancer.

In radioimmunotherapy (RIT), a highly specific pro-tumor substance is used as a carrier. Based on the specificity of the carrier and its affinity for the tumor, radionuclides are released in a targeted manner to focus their lethal effect on tumor cells. This is a non-toxic treatment method for the host(4,5). RIT is becoming a focus of study internationally. However, studies on RIT of ovarian cancer are limited (6). In the present study, a xenograft model of human OC-3-VGH ovarian cancer in nude mice was established to investigate the *in vivo* tumor inhibitory effect of ^{131}I-labeled ovarian cancer antigen 215 (^{131}I-CA215) antibody. This may provide the basis of further drug studies and a theoretical basis for the use of radiation therapy in the treatment of patients with estrogen-resistant ovarian cancer.

Materials and methods

Materials. The OC-3-VGH cell line derived from epithelial ovarian cancer tissue was supplied by Shanghai Guoyuan Biotechnology Co., Ltd. (Shanghai, China). The drugs (^{131}I-CA215 and CA215 antibodies) were supplied by Shanghai Guoyuan Biotechnology Co., Ltd. Trypsin, RPMI-1640, Trypan blue and a clean bench were purchased from Genetimes Technology, Inc. (Shanghai, China), Life Technologies

(Carlsbad, CA, USA), Zhejiang Tianhang Biological Technology Co., Ltd. (Hangzhou, China), Shanghai Shisheng Cell Biology Technology Co., Ltd. (Shanghai, China), and Shanghai Boxun Industry and Commerce Co., Ltd. (Shanghai, China), respectively.

Animals. Female BALB/c mice aged 6-8 weeks were purchased from the Shanghai Laboratory Animal Center of Chinese Academy Science (Certificate no. SCXK 2003-0002; Shanghai, China). Animals were housed in a laminar flow rack and fed with sterilized standard diets. Water and food were allowed *ad libitum*. The animal housing was well-ventilated at a temperature of 25±2˚C and a relative humidity of 40-70% under a 12-h light/dark cycle. The study was approved by the Shanghai Institute of Planned Parenthood Research (Shanghai, China) and the Animal Care and Use Committee, respectively.

Cell culture. The OC-3-VGH cells were removed from frozen storage in liquid nitrogen and placed into a water bath at 37˚C. The freezing tube was removed following thawing and the surface of the tube was wiped with an alcohol cotton ball for sterilization. The cells were transfered to the culture flask with added medium on a clean bench. Fetal calf serum was added into the culture medium at a concentration of 10%. The revivified OC-3-VGH cell line was washed twice in a 10-fold volume of RPMI-1640 by low-speed centrifugation. The supernatant was discarded. The cancer cells were resuspended in 10 ml RPMI-1640, and then incubated in a humidified incubator at 37˚C and 5% CO_2. The medium was changed the next day, and the cancer cells were cultured for 2-3 passages prior to inoculation.

Hypodermic inoculation of cancer cells. Ovarian cancer cells at the logarithmic growth phase were treated with 0.25% trypsin for dissociation for a short time, and then suspended in fresh culture medium. The suspension was centrifuged at 89.52 x g for 5 min. The cell pellets were resuspended in phosphate-buffered saline checked with Trypan blue for cell viability (≥90%) and adjusted to $2x10^7$ cells/ml. Each female BALB/c mouse was inoculated hypodermically to the cervical spinal region (near the armpit) with 0.2 ml cell suspension. The formation of a skin mound indicated successful inoculation.

Establishment of an ovarian cancer model with cancer tissue sections. The tumor was removed from the tumor-bearing mice under aseptic conditions. Well-grown tumor tissue without liquefactive necrosis was used for the experiments. The tumor tissue was minced into 1x1x1 mm sections and transplanted into one side of the armpit (close to the galactophore) of other nude mice using a no. 20 trocar needle (Beijing Baichuan Tongxin Medical Equipment Co., Ltd., Beijing, China) within 1 h.

Grouping of transplanted subcutaneous tumor model mice. Following transplantation, when the tumors reached a volume of 100-300 mm^3, a total of 42 tumor-bearing nude mice were divided randomly into seven groups (each n=6). These comprised the negative control (NC) group treated with isovolumetric normal saline, the positive control (PC) group treated with 60 mg/kg cyclophosphamide, the high-dose CA215 antibody group (HA; 10 mg/kg), low-dose CA215 antibody group (LA; 2 mg/kg), high-dose ^{131}I-CA215 antibody group (^{131}I-HA; 10 mg/kg + 125 μCi), medium-dose ^{131}I-CA215 antibody group (^{131}I-MA; 6 mg/kg + 75 μCi;) and low-dose ^{131}I-CA215 antibody group (^{131}I-LA; 2 mg/kg + 5 μCi). All test drugs were injected intraperitoneally every day for 14 days in an isotopic laboratory. The weight of each mouse and the tumor volume were measured on days 0, 4, 8, 12 and 15, and the survival time of the mice was also recorded.

Relative tumor growth rate. With the formation of tumor nodules, the tumor volumes were calculated by measuring the tumor length (a) and width (b), and by using the formula $V = 1/2 \times a \times b^2$ to determine the growth curve. The relative tumor volume (RTV) was calculated by $RTV = V_t/V_0$, where V_0 is the tumor volume prior to drug administration and Vt is the tumor volume measured at a given time following drug administration. Antitumor activity was evaluated by the relative tumor increase rate (T/C) using the following formula: T/C (%) = TRTV/CRTV x 100, where TRTV is the RTV of the treatment group and CRTV is the RTV of the negative control group. The therapeutic efficiency was evaluated based on the following criteria: T/C >40% indicated no therapeutic effect, whereas T/C ≤40% with $P<0.05$ indicated a positive therapeutic effect.

Tumor inhibition rate (TIR). The nude mice were sacrificed by decapitation on day 15, and the tumors were dissected and weighed. The TIR was calculated by comparing the weights of the transplanted tumors of the treatment group with those in the negative control group, using the following formula: TIR (%) = (1 - mean weight of the transplanted tumor of the treatment group/mean weight of the transplanted tumor of the negative control group) x 100.

Statistical analysis. Data are expressed as the mean ± standard deviation. Data were analyzed using SPSS version 11.0 for Windows (SPSS, Inc., Chicago, IL, USA). Multiple test groups in the antitumor experiments were compared with one-way analysis of variance using the post hoc Tukey test. Values of $P<0.05$ and $P<0.01$ were considered statistically significant.

Results

Cell culture. The revivified cells were observed to be attached, stretched and roughly spherical on the first day after cell recovery. Cells grew actively the following day, and covered the bottom of the bottle, requiring passage on the third or fourth day (Fig. 1).

Establishment of the animal model. Approximately seven days after the $2x10^7$/ml ovarian cancer cell suspension was inoculated subcutaneously into the nude mice, a protuberance was observed on the back and neck of all nude mice (Fig. 2A). The tumor formation rate was 100%. The tumor growth curve indicated that the tumor grew exponentially with time. The tumor growth velocity was considerably enhanced following the 35th day. Compared with the tumor volume on the seventh day, a significant increase in volume occurred after 42 days ($P<0.05$; Table I).

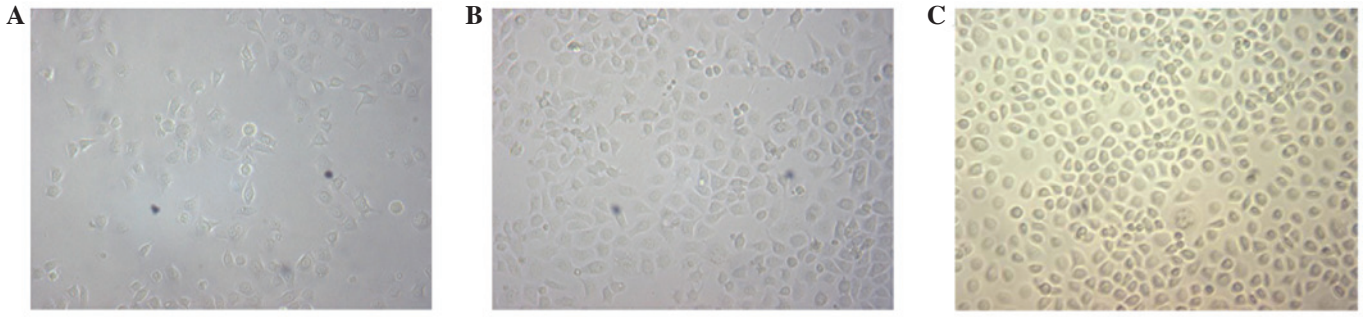

Figure 1. OC-3-VGH cell culture. Cell growth on the (A) first, (B) second and (C) fourth days (magnification, x400).

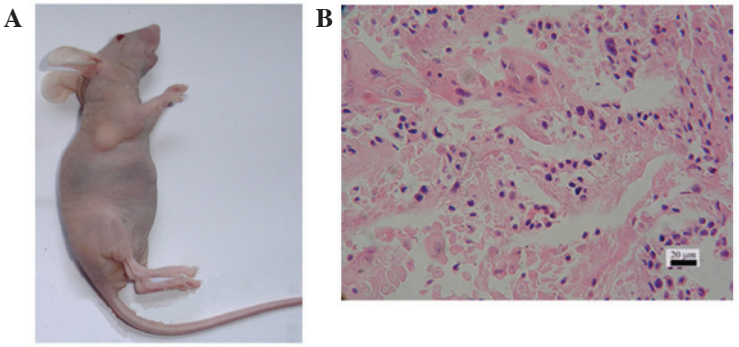

Figure 2. Establishment of a model of OC-3-VGH ovarian cancer in nude mice. (A) Subcutaneous tumor. (B) Tumor slide stained by hematoxylin and eosin..

Two months after inoculation, the tumor was dissected and found to be gray-white. The tumor tissues were embedded in parrafin (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), cut into sections (4 μm thick) and processed by hematoxylin and eosin staining (Zhuhai Baso Biotechnology Co., Ltd., Zhuhai, China). Histological examination using an inverted microscope (Motic AE31; Speed Fair Co., Ltd., Richmond, Canada) revealed that the tumor cells were large in volume, and the nuclei were large and strongly stained. A large amount of nuclear fission occurred and atypia was significant. No evident normal ovarian tissue was visible (Fig. 2B). In other pathological sections of the mouse organs, including the liver, lung, kidney and axillary lymph nodes, the structure was clear and the tissues were complete. No tumor cells were observed, indicating that the transfer to these organs did not occur.

Effect of ^{131}I-CA215 antibody on the body weights of nude mice bearing OC-3-VGH tumors. All tumor-bearing mice survived during the administration period, and their weight increased to different degrees (Table II). Compared with that prior to the administration (day 0), at post administration (day 15) the weights of the mice in the NC, PC and ^{131}I-LA groups showed significant increases (P<0.01). The weight in the LA and ^{131}I-MA groups also increased and the differences were statistically significant (P<0.05). The weights of the nude mice of all groups on day 15 showed no statistically significant differences (P>0.05).

Inhibitory effect of ^{131}I-CA215 antibody on the volume of OC-3-VGH tumors implanted in nude mice. Prior to administration (day 0), the tumor volume of each drug-treated group showed no statistically significant difference compared with that of the NC group (P>0.05). On the second day after administration, the tumor volume in the PC, HA, ^{131}I-HA and ^{131}I-MA groups was clearly reduced compared with that of the NC group (P<0.05). The tumor growth rate in the PC and all treatment groups was slow, and the RTV in the PC, HA, LA, ^{131}I-HA and ^{131}I-MA groups was significantly reduced compared with that of the NC group (P<0.05 or P<0.01). The results are shown in Table III. On day 15, the T/C of each treatment group was greatly reduced. The T/C values of the HA, ^{131}I-HA and ^{131}I-MA groups were 54, 30, and 48%, respectively. With the exception of the ^{131}I-LA group (P>0.05), the remaining groups showed a significant difference (P<0.01). Although the T/C of the ^{131}I-LA group was higher than that of the LA group on day 15, the decline of the T/C in the LA group at each time point was not significant (P>0.05).

Inhibitory effect of ^{131}I-CA215 antibody on the weights of OC-3-VGH tumors implanted in nude mice. The results of the *in vivo* antitumor experiment demonstrated that, compared with the NC group, the PC, HA and LA groups exhibited inhibition of the growth of the solid OC-3-VGH tumors (P<0.05). An increase of the monoclonal antibody dose enhanced the antitumor effect. The tumor inhibition rate of HA was 33.59% (higher than that of the PC group), indicating that the tumor growth was significantly inhibited (P<0.01). ^{131}I-HA, ^{131}I-MA and ^{131}I-LA treatment also inhibited the growth of solid OC-3-VGH tumors compared with those in the NC group. In addition, with the increase of the monoclonal antibody dose and ^{131}I content, the antitumor effect was enhanced. Among the

Table I. Weight and tumor volume of nude mice with tumors.

	Day							
Variable	7	14	21	28	35	42	50	57
Body weight (g)	19.23±0.93	22.83±0.61[a]	23.07±0.76[b]	23.30±0.95[b]	21.43±0.81[a]	23.57±1.53[b]	25.81±1.36[b]	25.30±1.31[b]
Tumor volume (mm^3)	6.73±0.56	21.31±4.60	36.19±5.73	37.12±14.28	41.08±22.91	89.19±45.30[b]	103.39±67.15[b]	291.34±202.22[b]

[a]P<0.05 and [b]P<0.01 compared with day 7. Data are presented as the mean ± standard deviation.

Table II. Body weight and TIR of nude mice bearing OC-3-VGH tumors following various treatments.

			Body weight (g)			
Group	Dose	^{131}I (μCi)	Day 0	Day 15	Tumor weight (g)	TIR (%)
NC	0.5 ml	-	19.35±0.83	22.26±0.84[a]	0.131±0.019	1.00
PC	60 mg/kg	-	19.25±0.73	21.79±1.06[a]	0.096±0.003[b]	26.72[c]
HA	10 mg/kg	-	20.64±1.27	22.55±0.95	0.087±0.007[d]	33.59[d]
LA	2 mg/kg	-	20.01±1.08	21.74±0.86[c]	0.102±0.017[b]	22.14[c]
^{131}I-HA	10 mg/kg	125	20.16±2.83	22.21±2.58	0.046±0.022[d,e]	64.89[d,e]
^{131}I-MA	6 mg/kg	75	20.10±0.74	22.36±1.26[c]	0.071±0.017[d]	45.80[d]
^{131}I-LA	2 mg/kg	25	18.00±0.79	21.00±0.96[a]	0.117±0.030	10.69

[a]P<0.01 and [c]P<0.05 compared with day 0; [b]P<0.05 and [d]P<0.01 compared with the NC group; [e]P<0.05 compared with the HA group. Data are presented as the mean ± standard deviation. TIR, tumor inhibition rate; NC, negative control; PC, positive control; HA, high-dose CA215 antibody; LA, low-dose CA215 antibody; ^{131}I-HA, high-dose ^{131}I-CA215 antibody; ^{131}I-MA, medium-dose ^{131}I-CA215 antibody; ^{131}I-LA, low-dose ^{131}I-CA215 antibody.

Table III. Effect of various treatments on tumor volume in nude mice bearing OC-3-VGH tumors.

Group	Tumor volume (mm^3)		RTV	T/C (%)
	Day 0	Day 15		
NC	1.43±0.14	160.82±20.07	112.74±15.86	100
PC	1.42±0.30	92.34±8.31[a]	67.42±16.38[a]	60[a]
HA	1.45±0.23	85.45±1.88[a]	59.98±10.14[b]	54[b]
LA	1.49±0.33	113.74±29.44	82.12±35.65[a]	71[a]
^{131}I-HA	1.35±0.13	47.03±15.93[a]	34.80±11.80[b]	30[b]
^{131}I-MA	1.34±0.27	71.39±12.42[a]	53.90±9.84[b]	48[b]
^{131}I-LA	1.53±0.38	136.02±43.12	88.31±10.69	80

[a]P<0.05 and [b]P<0.01 compared with the NC group. Data are presented as the mean ± standard deviation. NC, negative control; PC, positive control; HA, high-dose CA215 antibody; LA, low-dose CA215 antibody; ^{131}I-HA, high-dose ^{131}I-CA215 antibody; ^{131}I-MA, medium-dose ^{131}I-CA215 antibody; ^{131}I-LA, low-dose ^{131}I-CA215 antibody; RTV, relative tumor volume; T/C, relative tumor increase rate.

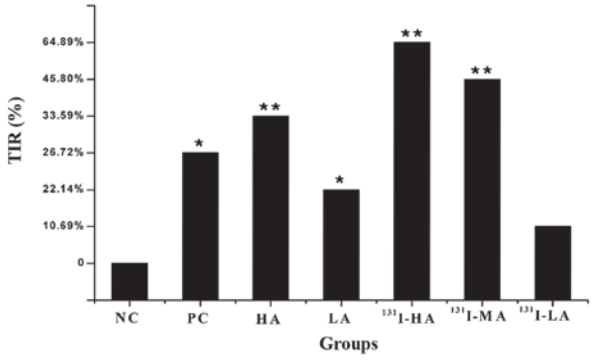

Figure 3. TIR in the various groups. *P<0.05 and **P<0.01 compared with the NC group. TIR, tumor inhibition rate; NC, negative control; PC, positive control; HA, high-dose CA215 antibody; LA, low-dose CA215 antibody; ^{131}I-HA, high-dose ^{131}I-CA215 antibody; ^{131}I-MA, medium-dose ^{131}I-CA215 antibody; ^{131}I-LA, low-dose ^{131}I-CA215 antibody.

groups, the tumor inhibition rates in the ^{131}I-HA and ^{131}I-MA groups were 64.89 and 45.80%, respectively, indicating a significant inhibition of tumor growth (P<0.01) and were higher than those in the PC and HA groups. Compared with the HA group, the ^{131}I-HA group exhibited an improved tumor inhibition effect and the difference was statistically significant (P<0.05). Compared with the LA group, the ^{131}I-LA group exhibited improved tumor inhibition effects, but the difference was not statistically significant (P>0.05). The results are shown in Fig. 3.

Discussion

The reduction of the mortality from ovarian cancer requires early detection so that the tumor can be controlled within the organ restrictions. At present, an effective treatment for advanced stage ovarian cancer is lacking (7,8). Currently, studies on ovarian cancer generally focus on its association with estrogen and its receptor (9,10). The research and development of new medicines for ovarian cancer treatment has concentrated on the pharmaceutical targeting of estrogen or its receptor (11). There are fewer studies on the types of ovarian cancer in which the relevance of the estrogen receptor is low. The OC-3-VGH cell line is a type of ovarian cancer that seldom expresses the estrogen receptor (3). The successful establishment of a model of this cell line in the present study further diversifies the animal models of ovarian cancer, and expands the pharmaceutical study of ovarian cancer. It provides a new foundation for studies of types of ovarian cancer that are resistant to clinical treatment with estrogen antagonists. In the present study, cancer cells were not found in organs such as the liver, kidney, lungs and axillary lymph nodes, indicating that the subcutaneous tumor that formed following the subcutaneous inoculation of OC-3-VGH cells did not transfer to the organs. Whether tumor cells can transfer and grow in animal hosts depends on the properties of the tumor cell (the seed), that is, the metastatic ability, as well as its organ tissue environment (the soil) (12,13). Firstly, the metastatic ability of tumor cells is crucial to the incidence of metastasis (14-16); tumor metastasis is a complex process. To grow in the host and trigger invasion and metastasis, tumor cells need the ability to damage the basilar membrane, infiltrate the connective tissue mesenchyme and invade the basement membrane of the small blood vessels and lymphatic vessels. In addition, tumor cells require the ability to penetrate through the immunological barrier of the host body. Furthermore, not all tumor cells can invade and transfer in the host body. Only those with high metastatic potential and certain numbers can generate the invasion and metastasis. However, further studies should be conducted to investigate whether the OC-3-VGH ovarian cancer cell line shows metastasis in other animal models. The establishment of an animal model of the OC-3-VGH ovarian cancer cell line provides a new approach to the establishment of animal models with human tumor cell lines and to developing a treatment for ovarian cancer.

In RIT, specific antibodies against tumor-associated antigens are used as a radionuclide carrier and labeled with radionuclide. The radionuclide-carrier complex is injected into the animal body to specifically bind with the corresponding antigens of tumor cells. Thus, a substantial quantity of radionuclide with a notable concentration gathers in the tumor tissue for a long time and the internal radiation of the tumor

body is realized. Therefore, the cytotoxic effect can be brought into play. A previous study applied this principle to radioimmunoimaging for the clinical diagnosis of tumors (17). RIT has presented clear effectiveness in the treatment of cancer in the blood system (18). However, due to reasons such as fewer vessels in solid tumors and an enlarged pressure on tissue space from hypoperfusion, the treatment of solid tumors with RIT continues to face great challenges. Andersson *et al* (19) used ^{211}At-Mx35 F $(ab')_2$ in clinical treatment via the intraperitoneal route of administration. A pharmacokinetic assay verified that it had certain effects in the treatment of miniature ovarian cancer, and patients did not present any evident untoward effects. In the present study, the radionuclide ^{131}I was bound with CA215 antibody and its killing effect on human OC-3-VGH ovarian cancer cells was investigated.

Although the simple application of antibodies can inhibit tumor growth to a certain degree, the effect is not significant. However, the combination of antibodies and radionuclides can play a notable role in the killing of tumor cells. The results of this study demonstrate that cyclophosphamide, different doses of the antibody and ^{131}I-antibody can inhibit tumor growth in mice bearing human OC-3-VGH ovarian cancer. As shown in Fig. 3, on day 15, when the tumor inhibition rates of the various groups were ranked in descending order, this order was: ^{131}I-HA > ^{131}I-MA > HA > PC > LA > ^{131}I-LA > NC.

The efficacy of the ^{131}I-labeled antibody on the tumors was found to be dose-dependent. On day 15, the T/C ratio decreased as the ^{131}I-CA215 antibody dose increased over the tested dose range, and the T/C of ^{131}I-LA was higher than that of LA. Yet, the T/C of ^{131}I-LA was significantly lower on day 15 compared with the NC group. LA with the same antibody concentration showed no statistically significant difference at different time points, which may be attributed to the *in vivo* radiation effect of ^{131}I-LA. The above results indicate that with labeling of the antibody with ^{131}I, at the same dose of antibody, improved effects in inhibiting tumor cell increase are exhibited. In addition to immunobinding, this can also be attributed to the radiation effect of the radionuclide. Experiment in the current study verified that although tumor cells in certain organs cannot absorb ^{131}I, the administration of large doses of ^{131}I can lead to the shrinking of tumors and the relief of symptoms. This may be attributed to the gathering of ^{131}I in healthy thyroid cells, which irradiated the adjacent nidus. However, the mechanism requires further exploration.

In the present study, the tumors of the nude mice were not completely relieved, which may be associated with the large tumor volume at the beginning of treatment and the ^{131}I concentration in the tumor. A previous study verified that during targeted treatment with a radionuclide-labeled monoclonal antibody, sufficient radionuclides for the monoclonal antibody are required (20). When the tumor volume is relatively large, the pressure in the tumor will be raised, and the antibodies that are able to reach the tumor site, as well as the dose of radionuclide, will be reduced, and thus influence the efficacy. This indicates that RIT cannot be used alone in the treatment of ovarian cancer, and that combined treatment with other approaches or the use of RIT treatment for mini-tumors is more realistic. Harris *et al* (21) carried out experiments with RIT combined with traditional chemotherapeutics, which generated a satisfactory outcome. Therefore, the next area of study will be the inhibitory effect of I^{131}-CA215 antibody combined with traditional chemotherapeutics on human OC-3-VGH ovarian cancer cells, which is expected to provide a theoretical basis for radiotherapy in patients with estrogen-resistant ovarian cancer.

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