Comparison of two kinds of orthotopic xenograft models for human ovarian cancer

Genhai Zhu[1], Lan Hong[1], Shengtan Wang[1], Junhong Cai[3], Zhaoxin Yang[2], Maozhong Yao[2]

[1] Department of Gynecology, Hainan Provincial People's Hospital, Haikou; [2] Drug Safety Evaluation Center, Hainan Medical College, Haikou; [3] Research Center, Hainan Provincial People's Hospital, Haikou (China)

Summary

Objective: The aim of this study was to compare the characteristics of two orthotopic xenograft models established with human epithelial ovarian cancer solid tumor tissue slices and human ovarian carcinoma cell line OVCAR-3. *Materials and Methods:* Tumor tissues and cell line OVCAR3 of human epithelial ovarian cancer were grown in subcutaneous tissue and the subcutaneous tumor source was fetched and inoculated in ovarian capsule of nude mice under microscope to establish the orthotopic implantation model. At four and eight weeks after modeling, the orthotopic tumor formation rate, tumor diameter, metastasis rate outside the ovary, incidence rate of ascites, and CA125 levels in the two models were observed. *Results:* The orthotopic tumor formation rate in the solid tumor slices group (60.0%) was significantly lower than that in the cell line group (85.0%, $p < 0.05$). However, the tumor diameter, metastasis rate outside the ovary, incidence rate of ascites, and CA125 levels in the solid tumor slices group (2.4 ± 0.61 cm, 75.0%, 50.0%, and 80.13 ± 11.26 U/ml, respectively) were remarkably higher than those in the cell line group (1.6 ± 0.53 cm, 52.9%, 29.4%, and 36.5 ± 6.71 U/ml, respectively) ($p < 0.05$, respectively). *Conclusion:* There are differences between the two orthotopic xenograft models established with human epithelial ovarian cancer solid tumor tissue slices and human ovarian carcinoma cell line OVCAR-3. The biological characteristics of the solid tumor slices model are more similar to human ovarian cancer.

Key words: Human epithelial ovarian cancer; Nude mice; Ovarian orthotopic xenograft tumor; OVCAR-3.

Introduction

Ovarian cancer is a serious disease affecting women's health. Its mortality rate is the top one in the gynecological cancers although the surgery and treatments have been continually improving. The five-year survival of patients with ovarian cancer however has not yet been significantly increased [1]. Since nude mouse model of epithelial ovarian cancer is a basic tool for studying human ovarian cancer, establishing an orthotopic or metastatic human ovarian cancer animal model that has closer biological behavior to human ovarian cancer is of great significance for investigating the development of epithelial ovarian cancer and determining the efficacy of the treatment of epithelial ovarian cancer [2]. Animal model studies have made great progresses that a variety of models have been established, including spontaneous tumor model, xenograft model, drug-induced tumor model and gene model, according to the research goals [3]. Nowadays, the most commonly used animal model of ovarian cancer is the models established with tumor tissues or tumor cells, which can be divided into allograft and xenograft according to the source of the graft. Xenotransplantation is to transplant the human ovarian cancer cell line or tissue into the immunodeficient animals [4]. The orthotopic tumor model established by xenotransplantation is widely used for studying the ovarian cancer in recent years. Tumor grown *in situ* in the nude mice can better maintain their histological characteristics and potentials of the intraperitoneal metastasis so that they can accurately reflect the clinical course of epithelial ovarian cancer. Until now, most of the animal models were established by intraperitoneal perfusion or orthotopic transplantation with human ovarian cancer cells. However, the model of orthotopic implantation with tumor tissue directly was seldom studied [5]. To establish a model that can reflect the true pathophysiological processes *in vivo* is a guarantee for the reliable experimental data and results. Different models show different biological characteristics because of their tumor origin, cell passage number, and implanting parts. Although the model established with tumor cells is commonly used currently, the source and passage number of the tumor cell line could affect the performance of the tumor characteristics significantly [6]. Recently, the model established with tumor tissue was found having many advantages on the retention of biological characteristics of the tumor. Thus, the authors intend to compare two orthotopic xenograft models established with human epithelial ovarian cancer tissue slices and human ovarian carcinoma cell line OVCAR-3 in nude mice, in order to establish a nude mice model with more efficient and closer to the biological characteristics of human epithelial ovarian cancer.

Materials and Methods

Materials

Human epithelial ovarian cancer tissue was sampling from the specimen of a patient (52 years-old) who was pathologically diagnosed with poorly differentiated mucinous ovarian papillary adenocarcinoma in the clinical Stage IIIc. This study was con-

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Table 1. — *The characteristics of the two orthotopic xenograft models.*

Groups	Number	Orthotopic tumor formation rate (n, %)	Mean tumor diameter (cm)	Metastasis rate (n, %)	Incidence rate of ascites (n, %)
Tissue slices	20	12 (60.0)	2.4 ± 0.61	9 (75.0)	6 (50.0)
OVCAR-3 cell	20	17 (85.0)	1.6 ± 0.53	9 (52.9)	5 (29.4)
		$\chi^2 = 4.27$	$t = 2.28$	$\chi^2 = 4.09$	$\chi^2 = 3.92$
		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

ducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Hainan Provincial People' Hospital. Written informed consent was obtained from all participants.

Four to six weeks old female BALB/c nude mice, weighing 16.5 ± 3.21 g, were offered by the Experimental Animal Center of Guangdong Province, China. All animals were housed in a special pathogen free (SPF) room with constant temperature at 25°C and humidity at 45%. All of the feed and bedding materials were sterilized and the persons and goods underwent a rigorous microbiological control before they entered the laboratory. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Hainan Provincial People' Hospital.

Cell culture

Human ovarian carcinoma cell line OVCAR-3 was resuscitated and suspended in RPMI 1640 medium, followed by low-speed centrifugation. Then the cells were resuspended in RPMI 1640 medium supplemented with 10% FBS, penicillin (200 units/ml), and streptomycin (200 units/ml) and incubated at 37°C in an atmosphere of 5% CO_2/95% air/100% humidity for passage.

Preparation of tumor source with OVCAR-3

The tumor source derived from OVCAR-3 cells was prepared following the method described previously [7]. Briefly, OVCAR-3 cells in the exponential growth phase were harvested and rinsed with fetal bovine serum (FBS)-free RPMI 1640 medium. After centrifugation, cells were resuspended in phosphate buffered saline (PBS) in a cell density of 2×10^7/ml. Then 0.2 ml of the cell suspension per mouse was inoculated into ten nude mice subcutaneously at the back of the neck near the armpit. Four to eight weeks later, the tumor formation rate was 90%. As the tumor grew to one cm diameter, it was taken out and confirmed pathologically, and then cut into pieces in 1×1×1 mm.

Preparation of tumor source with solid tumor tissue slices

Fresh ovarian cancer tissue, rinsed with ice-cooled normal saline, was cut into tissue slices (size, 1×1×1 mm) on ice and placed in a sterile test tube. After nude mouse was anesthetized, the ovarian cancer tissue slices were implanted through a small incision made at the back of the neck of the nude mouse, and then the skin was sutured. The tumor formation rate was 50% at four to eight weeks after the operation. When the tumor size grew to one cm, it was taken out and then confirmed pathologically. Afterwards, the tumor was cut into pieces in 1×1×1 mm. There were also ten nude mice used for preparation of the tumor source with solid tumor tissue slices.

Establishment of orthotopic xenograft model

Orthotopic xenograft models were established as described previously [8]. In brief, after the mouse was anesthetized with 45 mg/kg body weight of 1% sodium pentobarbital, the small tissue block was incubated under the envelope of the left ovarian under the microscope and then OB glue was trickled on the surface of the tissue block. As the OB glue solidified, the ovarian was put back into the abdominal cavity and the subcutaneous layer and skin of mouse were sutured with No. 0 silk suture. Twenty nude mice were used for each group. At four weeks and eight weeks after the operation, ten mice of each group were sacrificed, respectively. The tumor size, bilateral ovarian involvement, ascites, and abdominal extent of implanting of each mouse were recorded. Part of the tumor tissue was used for pathological examination.

Determination of serum CA125

The blood of each mouse was collected by eyeball enucleation before dissection and subsequently centrifuged for ten minutes at 2,000 rpm after standing for 30 minutes at room temperature. Then the serum of each sample was harvested for determination of serum CA125 concentration using chemiluminescence immunoassay.

Statistical analysis

The authors compared the mean differences by the Student *t*-test and the rates by the chi-square test. Statistical analysis was conducted by SPSS 13.0 software, and $p < 0.05$ was considered statistically significant.

Results

Characteristics of the two orthotopic xenograft models

The subcutaneous tumor formation rate of the solid tumor tissue slices group was 40% (4/10). It was significantly lower than that of the OVCAR-3 cell line group (90% (9/10), $p < 0.05$). The orthotopic tumor formation rate of the solid tumor tissue slices group was remarkably lower than that of the OVCAR-3 cell line group (60% *vs.* 85%, $p < 0.05$). Nevertheless, the mean tumor diameter, the metastasis rate outside the ovary, and the incidence rate of ascites of the solid tumor tissue slices group (2.4 ± 0.61 cm, 75.0% and 50.0%, respectively) were prominently higher than those of the OVCAR-3 cell line group (1.6 ± 0.53 cm, 52.9% and 29.4%, respectively; $p < 0.05$) (Table 1, Figures 1, 2).

Serum CA125 concentration

The mean serum concentration of CA125 in the solid tumor tissue slices group was 80.13 ± 11.26 U/ml, significantly higher than that in the OVCAR-3 cell line group (36.5 ± 6.71 U/ml, $t = 2.57$, $p < 0.05$).

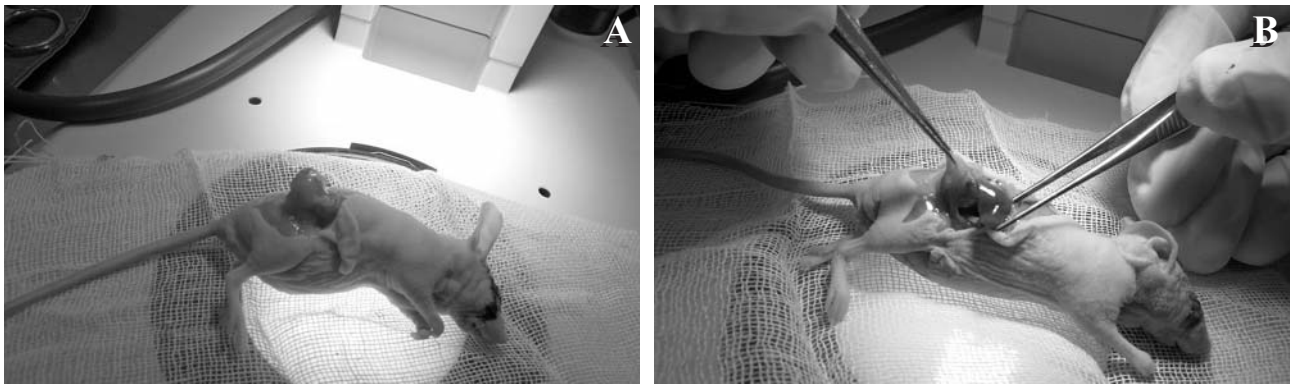

Figure 1. — The gross observation of the two models. A: The representative of mice in the OVCAR-3 cell line group; B: The representative of mice in the solid tumor tissue slices group.

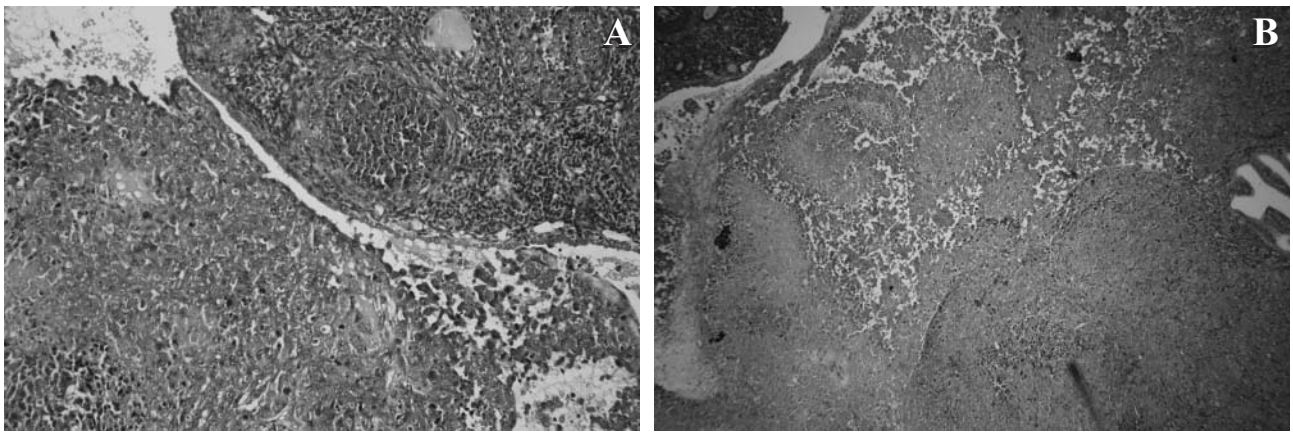

Figure 2. — The histopathological changes (HE staining, ×40) of the two models. A: The representative of mice in the OVCAR-3 cell line group; B: The representative of mice in the solid tumor tissue slices group.

Discussion

The nude mice models for human epithelial ovarian cancer are typically established with human ovarian carcinoma solid tumor tissues or human ovarian cancer cell lines. According to the implantation site, the models can be divided into subcutaneous tumor model, abdominal xenograft model and orthotopic xenograft model for different purposes [9, 10]. The cell line-derived subcutaneous xenograft model has several advantages, such as high tumor formation rate, relatively simple operation, and easy to observe and dissect, which is more appropriate for the studies merely on the tumor itself [11]. However, the tumors in the subcutaneous xenograft model rarely metastasize, which is too limited to reflect the overall physiological and pathological changes in human epithelial ovarian cancer. Thus, it is not suitable for the *in vivo* studies [12]. In contrast, the characteristics of the orthotopic xenograft model are closer to the *in vivo* growth pattern of human ovarian cancer, so that it can provide for more comprehensive results. Currently, the orthotopic xenograft model used was mainly derived from cell lines with different biological characteristics. Due to its high rate of tumor formation, it has been widely used in the clinical and basic research on human ovarian cancer [13]. However, the incidence rate of ascites and the metastasis rate are low in the orthotopic xenograft model derived from cell lines. If implanting the cells directly by intraperitoneal injection, the metastasis rate can be improved, but it does not reflect the occurrence and development of human ovarian cancer [14]. The ovarian carcinoma OVCAR-3 cell line used in this study was derived from poorly differentiated serous adenocarcinoma. Its metastasis rate outside the ovarian was only 52.9%, which has certain limitations if being used for the studies targeted to the tumor metastasis.

The orthotopic xenograft model established with the poorly differentiated carcinoma tissue slices has more similar biological characteristics to human ovarian cancer. For instance, it has high incidence rate of ascites and metastasis rate, its tumor grows rapidly, and its serum CA125 level

is significantly higher than that of the models established with cell lines. Regarding this, it is an ideal model for the overall studies on human ovarian cancer. However, such model has low rate of tumor formation, which was reported being only 28% to 40% in China [15, 16]. The authors believed that improving the tumor formation rate is the key for establishing successful model. Thus, before orthotopic transplantation, a transitional phase of subcutaneous tumor formation was necessary for either the cell lines-derived orthotopic xenograft model or the solid tumor tissue slices model. This was an adaptation process, i.e. it was an *in vivo* screening process in nude mice. The subcutaneous tumor tissue slices were then transplanted into nude mice *in situ* and consequently the tumor formation rate was significantly increased [17, 18]. The tissue slices for implantation should not be too thick (one mm was more suitable) and they should be placed smoothly on the wound, so that it was easy to establish new blood vessels. The orthotopic xenograft model also can be prepared by injecting the cells (derived from the cell lines or the cell suspension prepared from solid tumor tissue) directly into ovarian envelope [19, 20], but it requires too much time and the cell suspension cannot be stored for too long. Thus, the orthotopic xenograft model established by injection of cell suspension is only suitable for preparations of a small number of models. Conversely, the implantation of tissue slices is relatively simple and more suitable for preparing a large number of models.

In summary, the characteristics of the two orthotopic xenograft models established with solid tumor tissue slices and OVCAR-3 cell line were quite different. The former is closer to the biological behavior of human ovarian cancer but has low rate of tumor formation. With the passage *in vivo* in nude mice and the processing of tissue slice, the rate of tumor formation is expected to increase remarkably.

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Address reprint requests to:
GENHAI ZHU, M.D.
Department of Gynecology,
Hainan Provincial People'Hospital,
Hainan 570311 (China)
e-mail: genhaizhu@126.com