The diagnostic value of color Doppler ultrasound on ovarian cancer in nude mice

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Summary

Objective: Color Doppler ultrasound was used to evaluate the intraabdominal ovarian cancer tumor model in nude mice, to explore its diagnostic value on ovarian cancer in nude mice. Materials and Methods: A SKOV3 cell line was utilized to establish intraperitoneal implant tumor model of ovarian cancer in nude mice. The nude mice were divided into five groups, each group received different drugs. Color Doppler ultrasound was used to evaluate the size of intraabdominal tumor and its color Doppler blood flow. And the final pathological results were compared with the results of color Doppler ultrasound. Results: Color Doppler ultrasound can evaluate the change of length diameter (a) and width diameter (b) of tumor tissue over time and the change of echo of tumor tissue in nude mice; the blood supply and vascular resistance index (RI) value detected by color Doppler ultrasound showed no significant difference; the length diameter, width diameter of tumor measured by ruler, and color Doppler ultrasound showed no significant differences (p > 0.05). Conclusion: Color Doppler ultrasound is of important diagnostic value for ovarian cancer in nude mice. And it is the preferred method to evaluate the growth of intraabdominal implantation tumor in nude mice.

Key words: Color Doppler ultrasound; Nude mice; Ovarian cancer.

Introduction

Ovarian cancer is one of the most common malignancies of the gynecological system and the mortality of it was highest among all gynecologic ones [1, 2]. It is the leading cause of death from gynecological diseases among women worldwide [3, 4], and it poses a serious threat to women's health [5]. In addition to genetic factors, environmental factors such as obesity and smoking are also associated with an increased risk of ovarian cancer [6]. There is also a negative correlation between age of menarche and the onset risk [7]. The subtype of ovarian cancer can be histologically differentiated [8]. Among them, malignant ovarian cancer from the epithelium is the most common [9]. Different subtypes of ovarian cancer have different etiologies and different sensitivities to treatment [10]. Early stage ovarian cancer lacks obvious clinical manifestations and diagnostic methods, therefore nearly 80% of the patients with ovarian cancer are found in the late stage. The five-year survival rate of advanced ovarian cancer patients is less than 30%, in patients whose tumors were confined to the ovaries themselves. The survival rate is significantly increased, so early diagnosis is very important for the treatment and prognosis of ovarian cancer. Color Doppler ultrasound is currently the most important examination method for the diagnosis of gynecological diseases. It can also be used as an effective means to evaluate the efficacy of drugs and surgery. At present, there are few studies on the abdominal tumor model of nude mice using color Doppler ultrasound at home and abroad. This study aims at investigating the value of color Doppler ultrasound in evaluating the growth status and blood supply of intraabdominal ovarian cancer in nude mice.

Materials and Methods

Human ovarian adenocarcinoma cell line SKOV3 was selected. Forty BALB/C nu/nu female nude mice, aged 4-5 weeks, were raised in a SPF environment. An IU22 ultrasonic diagnostic instrument was used, and the probe frequency was 3.5-8 MHz. SKOV3 cells were placed in 5% CO2 incubator, at a 37°C constant temperature, with a RPMI-1640 medium plus 10% fetal bovine serum. The logarithmic growth phase cells were taken and the cell concentration was adjusted to 1×10⁷/ml.

The construction of models of abdominal implantation tumor in nude mice: 0.2 ml SKOV3 cells with the concentration of 1×10⁸/ml were injected into the abdominal cavity of nude mice, to observe whether there were redness, infection, ulceration, spillage at the injection site, abdominal distension, and systemic cachexia such as emaciation daily. Five groups of nude mice were treated with normal saline, 25 mg/kg Gleevec, 50 mg/kg Gleevec, 5.0 mg/kg cisplatin single drug therapy, and 25 mg/kg Gleevec+5.0 mg/kg cisplatin combined therapy. At 7, 14, 21, and 28

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days after intraperitoneal injection of SKOV3 cells, the tumor size, tumor blood supply, and vascular resistance index (RI) were evaluated by color Doppler ultrasound. The size, shape, and internal echo of tumor in nude mice were observed. The long diameter (a) and short diameter (b) of the tumor were measured. And color Doppler flow imaging (CDFI) technique was used to observe the blood flow distribution of tumor in abdominal cavity of nude mice, and measuring the vascular RI in the lesion.

**Results**

Tumors in the abdominal cavity of nude mice were examined by color Doppler ultrasound every seven days (i.e., the 7th, 14th, 21st, and 28th days of the experiment). Ultrasonography showed that the tumor tissues in the abdominal cavity of nude mice were irregular or nearly round. The tumor boundary was clear, and the tumor itself had uniform echogenicity. Color Doppler ultrasound detected the length diameter (a) and width diameter (b) of tumor tissue in the abdominal cavity of nude mice in each group that gradually increased with time (Table 1). Color Doppler ultrasonography can also detect the changes of tumor echo in the abdominal cavity of nude mice in each group. The tumor tissues of nude mice in each group gradually developed from hypoechoic to isoechoic. The internal echo gradually developed from uniform to non-uniform, and the boundary between the tumor and surrounding tissues gradually became unclear.

Color Doppler ultrasound also detected the changes of tumor blood supply in the abdominal cavity of nude mice in each group. At the beginning of the experiment, stallate and punctiform blood flow signals could be seen in and around tumor tissues in the abdominal cavity of nude mice in each group. As the experiment progressed, color Doppler ultrasound detected the gradual appearance of stub and branching blood supplies within and around the tumor. By measuring the value of vascular RI, there was no significant change in the vascular RI of tumor tissues of nude mice in each group, with no significant difference (p > 0.05) (Table 2).

All of the nude mice in each group were sacrificed by spinal cord dislocation at the end of the experiment, just at the 7th day after the end of medication (i.e., the 28th day of the entire experiment). The abdominal cavity of the nude mice was split. Macroscopically, tumor masses of different sizes were observed in the abdominal cavity of each group of nude mice. Tumor masses in the abdominal cavity of each group of nude mice were completely removed. And the size of each tumor mass was measured accurately with a scaleplate. The volume of tumor tissue in abdominal cavity of each group was recorded. The results were compared with those measured by color Doppler ultrasound at the last time (i.e., the 28th day of the entire experiment). There was no significant difference in tumor length diameter, width diameter between scaleplate and color Doppler ultrasound measurements (p > 0.05) (Table 3).

**Discussion**

Ovarian cancer is the fifth leading cause of cancer death among American women and the most common cause of death among gynecologic malignancies [11]. The five-year survival rate of advanced ovarian cancer is only 29% and the five-year survival rate of tumors confined to ovary is more than 90%. Padilla et al.’s study clearly showed that palpation had a lack of sensitivity in the diagnosis of uterine and ovarian tumors [12]. Therefore, in the differentiation and treatment of gynecological diseases, ultrasound has become a widely used diagnostic method [13]. Color Doppler ultrasonography is an auxiliary examination method to diagnose gynecological diseases by adjusting the distribution of grayscale imaging and blood flow spectrum. Studies showed that benign and malignant tumors can be distinguished according to the relatively rich blood supply of malignant tumors [14]. In most ovarian cancer screening studies, the assess sensitivity of ultrasound was similar to the CA-125 level [15]. Therefore, ultrasound is one of the preferred screening methods for the diagnosis of ovarian cancer.

The mode of spread and metastasis of human ovarian cancer is intraperitoneal dissemination and metastasis. The abdominal tumorigenesis model established in this study can simulate the spread and metastasis of ovarian cancer in human body, which is more in line with clinical practice. Moreover, the nude mouse tumor model retained the pathological and biological characteristics of human tumors.

In this experiment, all of the nude mice were examined by color Doppler ultrasound. With high resolution, clear imaging, and sensitive color Doppler flow, the fine lesions

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**Table 1. — Intraperitoneal tumor size changes in each group of nude mice by ultrasound (mm).**

| Group  | a     | b     | a     | b     | a     | b     | a     | b     |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|
| A      | 1.31  | 0.45  | 0.92  | 0.47  | 3.89  | 0.91  | 3.53  | 0.78  |
| B      | 1.32  | 0.71  | 0.99  | 0.80  | 3.73  | 0.81  | 3.46  | 0.92  |
| C      | 1.28  | 0.55  | 0.87  | 0.61  | 3.70  | 0.99  | 3.38  | 0.82  |
| D      | 1.30  | 0.62  | 0.93  | 0.41  | 3.68  | 0.41  | 3.31  | 1.20  |
| E      | 1.33  | 0.57  | 0.87  | 0.40  | 3.60  | 0.69  | 3.15  | 0.48  |

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**Note:** The values are presented as mean ± standard deviation.
in the abdominal cavity of nude mice and its color Doppler blood flow distribution can accurately be detected. The tumor growth and blood supply in the abdominal cavity of nude mice in each group can be effectively monitored by measuring tumor size, observing tumor echo, and evaluating the changes of tumor blood flow distribution and vascular RI in the abdominal cavity of nude mice. And then the effect of different drugs on intraperitoneal ovarian cancer in nude mice was evaluated. This experimental study has shown that the growth pattern, echo, blood supply, and vascular RI of the transplanted tumor of human ovarian adenocarcinoma SKOV3 cell line inoculated in the abdominal cavity of nude mice were basically consistent with the ultrasonic characteristics of human ovarian cancer. In this experiment, color Doppler ultrasound was used to observe the echo of tumor tissue in the abdominal cavity of nude mice. The tumor tissue was hypoechoic or isoechoic. The inside echo of the tumor was uniform. Color Doppler ultrasonography can detect the blood flow distribution inside and around the tumor. The distribution of blood supply gradually developed from the star-shaped point at the beginning of the experiment to the short linear or branching blood supply distribution. During the experiment, the vascular RI of tumor tissues in the abdominal cavity of nude mice was detected weekly. The results showed that there was no significant difference in the vascular RI between groups. Due to the small size of the animals used in this experiment, namely nude mice, the abdominal cavity is not easy to form large tumor tissue. In addition, the duration of the experiment was only 28 days, which was relatively short. During this period, the tumor in the abdominal cavity of nude mice in each group only grew to a few millimeters. Therefore, the blood flow signals of tumor tissues in the abdominal cavity of the nude mice in each group were all extenuation, and the changes of vascular RI were not obvious. In the future, based on this experimental study, larger animals such as rabbits can be used as research objects and the study duration can be extended to further evaluate the angiogenesis of tumor tissues and the change of vascular RI. At the end of the experiment, all of the nude mice were sacrificed and tumor tissues in the abdominal cavity of each mouse were removed. Scaleplate was used for the actual measurement of tumor. And the actual value was compared with the last measurement of ultrasound in this experiment. Results showed that there was no significant difference between the length diameter and short diameter of the tumor measured by ultrasound and scaleplate. Statistical results showed no significant differences. The tumor size measured by ultrasound was equal to the actual measurement result, that is, the tumor size obtained by ultrasound was consistent with the actual measurement result. This study also showed that color Doppler ultrasound can accurately measure the size of tumor growth in abdominal cavity and is an important way for the diagnosis of abdominal tumor. It is of great significance for in vivo study of tumor. Therefore, color Doppler ultrasonography possesses important diagnostic value for ovarian cancer in nude mice and is the preferred method to evaluate the growth of enterocoelia tumor in nude mice.

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### Conflict of Interest

The authors declare no conflict of interests.


References

[1] Yahata T., Mizoguchi M., Kimura A., Orimo T., Toujima S., Kuninaka Y., et al.: “Programmed cell death ligand 1 disruption by clustered regularly interspaced short palindromic repeats/Cas9-genome editing promotes antitumor immunity and suppresses ovarian cancer progression”. Cancer Sci., 2019, 110, 1279.

[2] Siegel R.L., Miller K.D., Jemal A.: “Cancer statistics, 2016”. CA Cancer J. Clin., 2016, 66, 7.

[3] Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebeco M., et al.: “Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012”. Int. J. Cancer, 2015, 136, E359.

[4] Doufekas K., Olataita A.: “Clinical epidemiology of epithelial ovarian cancer in the UK”. Int. J. Womens Health, 2014, 6, 537.

[5] Kobayashi M., Rice G.E., Tapia J., Mitchell M.D., Salomon C.: “Exosomes are fingerprints of originating cells: potential biomarkers for ovarian cancer”. Res. Rep. Biochem., 2015, 5, 101.

[6] Beral V., Hermon C., Petre R., Reeves G., Brinton L., Marchbanks P., et al.: “Ovarian cancer and body size: individual participant meta-analysis including 25,157 women with ovarian cancer from 47 epidemiological studies”. PLoS Med., 2012, 13, 936.

[7] Gong T.T., Wu Q.J., Vogtmaenn E., Lin B., Wang Y.L.: “Age at menarche and risk of ovarian cancer: a meta-analysis of epidemiological studies”. Int. J. Cancer, 2013, 132, 2894.

[8] Rosen D.G., Yang G., Liu G., Mercado-Uribe I., Chang B., Xiao X., et al.: “Ovarian cancer: pathology, biology, and disease models”. Front. Biosci., 2009, 14, 2089.

[9] Permuth-Wey J., Sellers T.A.: “Epidemiology of ovarian cancer”. Meth. Mol. Biol., 2009, 472, 413.

[10] Cuellar-Partida G., Lu Y., Dixon S.C., Fasching P.A., Hein A., Burghaus S., et al.: “Assessing the genetic architecture of epithelial ovarian cancer histological subtypes”. Hum. Genet., 2016, 135, 741.

[11] Jemal A., Siegel R., Ward E., Murray T., Xu J., Thun M.J.: “Cancer statistics”. CA Cancer J. Clin., 2007, 57, 43.

[12] Romero-Laorden N., Oltos D., Fehm T., Garcia-Donas J., Diz-Padilla I.: “Circulating and disseminated tumor cells in ovarian cancer: a systematic review”. Gynecol. Oncol., 2014, 133, 632.

[13] Hua T., Ruijun G., Hongyan Z.: “Misdiagnosis analysis of pelvic mass by color doppler ultrasound”. Chinese Journal of Medical Ultrasound (electronic edition), 2011, 8, 2001. (In Chinese)

[14] Chun D., Yong G., Haining H., Haiyan X.: “Diagnostic value vascular resistance index combined with CA125 for ovarian tumor”. Journal of North Sichuan Medical College, 2017, 32, 788. (In Chinese)

[15] Menon-Andersen D., Mondick J.T., Jayaraman B., Thompson P.A., Blaney S.M., Bernstein M., et al.: “Population pharmacokinetics of imatinibmesylate and its metabolite in children and young adults”. Canc. Chemother. Pharmacol., 2009, 63, 229.

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