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Research Article

Expression of CK-7, CA125 and Survivin in Paraneoplastic Tissues at Different Distances from Orthotopic Human Epithelial Ovarian Carcinomas in Nude Mice

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Abstract

Objective: The objective of this study was to investigate the safety margin of ovary-sparing local tumorectomy by detecting the expression of CK-7, CA125 and survivin in paraneoplastic tissues at different distances from orthotopic human epithelial ovarian carcinomas in nude mice.

Methods: RT-PCR and IHC were used to measure the expression levels of CK-7, CA125 and survivin in the ovarian tissues of 20 normal nude mice as well as in the cancer tissue and paraneoplastic tissues (respectively 1 mm- 1.5 mm, 1.5 mm – 3 mm, 3 mm- 4.5 mm from the cancer) of 18 nude mice with orthotopic human epithelial ovarian carcinomas.

Results: According to the RT-PCR and IHC results, the expression levels or positive rates of CK-7, CA125 and survivin in the ovarian tissue 1 mm- 1.5 mm from the cancer were significantly higher than those in the normal tissue ($P<0.01$), but slightly lower than those in the cancer tissue with no statistical difference ($P>0.05$); the expression levels or positive rates in the ovarian tissue 1.5 mm – 3 mm from the cancer were significantly lower than those in the cancer tissue ($P<0.01$), but slightly higher than those in the normal tissue with no statistical difference ($P>0.05$); the expression levels or positive rates in the ovarian tissue 3 mm- 4.5 mm from the cancer were significantly higher than those in the normal tissue ($P<0.01$), but slightly lower than those in the cancer tissue with no statistical difference ($P>0.05$); there were no statistically significant differences in the expression levels or positive rates between the ovarian tissue 3 mm- 4.5 mm from the cancer and the normal ovarian tissue ($P>0.05$).

Conclusions: The expression levels or positive rates of CK-7, CA125 and survivin in the paraneoplastic tissues showed a decreasing trend towards the non-cancer side; the tissue 1 mm- 1.5 mm from the cancer may be an area with a risk of occult metastasis and 3 mm is the minimum safe distance for performing ovary-sparing local tumorectomy for orthotopic human epithelial ovarian carcinomas in nude mice.

Introduction

According to the age structure of patients with epithelial ovarian cancer, women of childbearing age, below 45 years of age, account for 21% and many of them have bilateral cancer. Many of these young patients strongly hope their fertility and ovarian endocrine function can be retained. In conservative surgeries, tumors without a complete capsule cannot be enucleated completely and can be treated only with ovary-sparing local tumorectomy. The reported fertility-sparing surgeries for epithelial ovarian cancer are mostly adnexectomy and enucleation of tumors with capsules [1,2]. Ovary-sparing local tumorectomy has only been reported in individual cases [3,4] and there is no definitely conclusive data. However, studies have shown that the mortality rate of patients with

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epithelial ovarian cancer treated with fertility-sparing surgery is higher than that of radical surgery [5] and that the recurrence rate of borderline ovarian tumors after being treated with tumor enucleation is 10.9%, significantly higher than that of patients undergoing adnexectomy [6]. There are many factors contributing to the above findings, among which surgical margin may be an important one. Currently, surgeons performing these surgeries generally determine the surgical margin by visual observation and experience, or by performing a rapid frozen examination of the margin. However, the coincidence rate between the result of the frozen section examination and the final pathological diagnosis is only 62% [7]. Therefore, both visual observation and frozen section examination are limited in determining the surgical margin. In order to retain the normal ovarian tissue to the utmost extent and ensure there is no residual cancer, we established a standard for screening tumor cell residues by detecting a series of molecular markers in tissues adjacent to epithelial ovarian cancer and concluded that negative expression of CK-7, CA125 and survivin can be used as the standard for excluding residual and occult cancer [8]. This study was designed to detect the expression levels of CK-7, CA125 and survivin in paraneoplastic tissues at different distances from orthotopic human epithelial ovarian carcinomas in nude mice through IHC and RT-PCR, investigate the paraneoplastic safe zone and minimum safe distance to determine the surgical safety margin at the molecular level and provide a basis for clinically accurate removal of tumor tissues and maximum preservation of normal ovarian tissues.

Materials and Methods

Materials

Cell Line: The human epithelial ovarian cancer cell line OVCAR3 was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Fetal bovine serum and RPMI-1640 medium were purchased from Invitrogen (CA).

Experiment Animals: Total of female BALB/c nude mice (4-6 weeks old and 15.9±3.19 g) were purchased from the Experimental Animal Center of Guangdong Province and were fed in the SPF (special pathogen free) barrier system. All the feed and bedding materials were sterilized. All the people and implements that entered the laboratory were subjected to strict microbial control.

IHC Reagents: Survivin: Rabbit Anti-Human Survivin McAb, No.: ZA-0530, Clone No.: EP119; CK-7: Mouse Anti-Human CK-7 McAb, No.: ZM-0071, Clone No.: OV-TL12/30; CA125: Mouse Anti-Human CA125 McAb, No.: ZM-0019, Clone No.: OC125; Secondary Antibodies: IHC Staining Kit, Concentrate DAB Kit. The above primary and secondary antibodies were purchased from Beijing ZSGB-BIO Co., Ltd.

Table 1: Primer sequences of CK-7, CA125 and survivin.

Primer	Lot No.	Positive-sense strand / Antisense strand	Length(bp)
β-actin	8400014061	AAGCAGGAGTATGACGAGTCCG	
	8400014060	GCCTTCATACATCTCAAGTTGG	559
survivin	8400014055	GCACTTTCTTCGCAGTTTCC	
	8400014054	GGACCACCGCATCTCTACAT	294
CA125	8400014063	GGTAGTAGCCTGGGCACTGT	
	8400014062	ACCCAGCTGCAGAACTTCAC	120
CK-7	8400014051	CAGGTGGTTATCCCGAAAGA	
	8400014050	GTTCCATTTGCAAAGGCTGT	102

RT-PCR Reagents: The reagent for total RNA extraction (the RNeasy Minikit Kit) was purchased from QIAGEN. RT-PCR reagents, primers and internal references were purchased from Shanghai Sangon Biological Engineering Co., Ltd. The PCR instrument was purchased from Leopard Scientific Instruments (Beijing) Co., Ltd. Primer sequences are shown in Table 1.

Methods

Cell Culture: Medium was added after the recovery of the cell line and the cells were centrifuged at a low speed. Then, the supernatant was removed and the cells were sub-cultured at 37°C and 5% CO_2 in the RPMI-1640 medium containing 10% new bovine serum, 200 u/ml penicillin and 200 u/ml streptomycin.

Using the cell line to prepare the tumor source: Logarithmic-phase OVCAR3 cells were treated with 0.25% trypsin and cells were suspended in serum-free medium. The cells were centrifuged and resuspended in phosphate-buffered saline (PBS) at a density of 2 x 10^6 cells /0.1 ml. Ten nude mice received 0.2 ml cell suspension each by subcutaneous injection in the armpit near the neck or back. The tumor formation rate was 90% after 4-8 weeks and the tumor bodies were removed after it grew to about 1cm in size and examined by pathological biopsy. Under sterile conditions, the tumor source was trimmed to be 1 mm x 1 mm x 1 mm tissue blocks.

Establishment of the orthotopic epithelial ovarian carcinoma model: 20 nude mice were treated with intraperitoneal anesthesia by 1% amyl barbital sodium (45 mg/kg body weight). A small tumor block was inoculated into the ovarian capsule at one side for each one of the mice and instilled with OB glue on the surface, which was in the abdominal cavity after solidification. Then, No. 0 silk suture was used to suture the subcutaneous layer and the skin.

All the mice were dissected after the implantation for 8 weeks and the ovary with the tumor was removed. The site 1 mm from the visually observed border of the cancer was used as the starting point and the adjacent tissues of equal size were finally obtained by using a ruler to make a mark every 1.5 mm towards the non-cancer side. Each section was made into 1.5 mm-thick tissue sheet, which was conserved in liquid nitrogen for further testing. Routine pathological biopsy was performed for the tissue at the cancer side of each of the paraneoplastic sections to exclude malignant tumor. 20 nude mice were inoculated with a success rate of 90%. At the same time, 20 nude mice, which underwent the same procedure but received an orthotopic transplantation of ovarian tissue from normal nude mice, were used as a control.

Semi-quantitative RT-PCR

Total RNA extraction: The frozen tissue was ground into powder in liquid nitrogen. According to the instructions of RNeasy Minikit, the chloroform-containing buffer was added for centrifugation. The supernatant was transferred to the pre-cooled centrifuge tube and cultured on ice for 5 min. Then, it was centrifuged at 4°C and 300×g for 2 min and the supernatant was added to the buffer containing β-Mercaptoethanol (β-ME), which was then vibrated violently for 15s. 96-100% RNA special alcohol was added and mixed thoroughly through repeated suction. After that, the suspension was transferred to the RNA spin column and centrifuged at 4°C and 8000×g for 15s. The RNeasy Mini spin column was retained, while the collection tube and the liquid in it were discarded. Then, RPE buffer was added and the suspension was centrifuged at 8000×g for 2 min. After replacing with a new collection tube, the suspension was centrifuged again at 8000×g for 1 min to thoroughly remove the alcohol in the buffer. RNase-free water was added to rinse the spin column at 8000×g for 1 min to collect RNA and it can be rinsed twice. Residual DNA was eliminated by Dnase digestion and the concentration and purity of

RNA were detected by using an ultraviolet spectrometer. To detect any DNA contamination, PCR amplification was performed by using β-Actin primers. 50 ng RNA that was electrophoresed on 1% agarose gels and ultraviolet light was used to detect and photograph. The OD value of total RNA was measured and the concentration was determined.

Synthesis of cDNA: 5 μl DEPC H2O, 1 μl oligo, 1 μl 10mM dNTP mixed liquor and 4 μg RNA were added successively. It was cultured at 65°C for 5min and then rapidly moved to the ice surface to cool down. After adding the mixed liquor containing DTT and RNase inhibitor, it was cultured at 42°C for 2 min. After adding 1 μl synthetase (Superscript II), it was cultured at 42°C for 50 min, then heated at 70°C for 15 min and reserved at 4°C.

DNA amplification: The semi-quantitative PCR reaction system was 25 μL.

10×PCR buffer 2.5 μl

10 mM dNTPs 1.0 μl

50 mM $MgCl_2$ 2.0 μl

Taq DNA polymerase 0.3 μl

20 μM β-actin up 1.0μl

down 1.0μl

20 μM primer up 1.0μl

down 1.0μl

DEPC H2O 12.2μl

cDNA 3μl

The PCR conditions are as follows. Survivin: 94°C denaturation for 1min, 56°C annealing for 1 min and 72°C extension for 2 min; CA125: 95°C denaturation for 1min, 48°C annealing for 1 min, 72°C extension for 1 min; CK-7: 94°C denaturation for 30s, 55°C annealing for 30s, 72°C extension for 30s. All reactions had a final extension at 72°C for 5 min after 35 cycles and the products were stained by ethidium bromide. Automated gel imaging analyzer was used to analyze the result of 2% agarose gel electrophoresis to detect the integrated optical density (IODs). Then, the IOD ratio of target and reference genes, i.e. the relative amount of the target genes' mRNA, was calculated. The relative amount > 0.5 was considered to be positive.

IHC Assay: The two-step IHC assay was performed according to the kit instructions. The ovarian cancer tissue was used as a positive control and PBS, instead of the primary antibody, was used as a negative control.

The paraffin section was routinely dewaxed and dehydrated; xylene dewaxing was performed for 3 times each for 10 min; gradient alcohol dehydration (100%, 95%, 80% and 70%) was performed for 3 times each for 5 min. After rinsing with tap water for 3 times, PBS rinsing was performed for 3 times each for 5 min. 50μl peroxidase blocking solution was added and the section was incubated for 15 min at room temperature. Then, it was rinsed with PBS buffer for 5 min×3 times. After blotting up PBS buffer, 50 μl normal sheep serum was added and the section was enclosed at 37°C for 20 min. Then, the serum was blotted up without rinsing. Microwave antigen retrieval: the section was placed in the citric acid buffer (PH6.0), heated by medium-high microwave for 7 min and then cooled down at room temperature. The section was rinsed with PBS buffer for 5 min×2 times and incubated overnight with the primary antibody at an appropriate dilution at 4°C. Then, it was rinsed with PBS buffer for 5 min×3 and incubated with the HRP-labeled secondary antibody for 30 min. The section was then rinsed with PBS buffer for 5 min×3 and stained with freshly prepared DAB solution until the positive brownish yellow staining was observed under the microscope (5 min). After that, the section was rinsed with tap water, counterstained with hematoxylin for 5 minutes and reacted in 1% hydrochloric acid alcohol for 5s. Then, the section was rinsed with tap water and soaked in 1% ammonia for 20s. Gradient alcohol dehydration (70%, 80%, 95% and 100%) was performed for 2 min at each concentration, followed by xylene transparentizing for 5 min and neutral balsam mounting.

Scoring Criteria for IHC Results [9]

Positive expression shows as brownish yellow granules taking the place of cytoplasm, cell membrane, or nucleus. The expression of CK-7 is mainly located in cytoplasm; the expression of CA125 is mainly located in cytoplasm or cell membrane; the expression of survivin is mainly located in nucleus or cytoplasm. 5 high power fields were observed randomly for each section. Reference Standard: Semi-quantitative treatment should be performed for comprehensive staining intensity and positive cell proportion under high power microscope. Scoring criteria for staining intensity are as follows: non-staining – 0; yellow – 1; brownish yellow – 2; dark brown – 3. Scoring criteria for positive cell proportion are as follows: <10% - 0; 10%-40% - 1; 40%-70% - 2; ≥70% - 3. The final results were obtained by adding the two scores together and the grading criteria are: 0-1 (-); 2 (+); 3-4 (2+); 5-6 (3+).

Statistical Analysis

All the data was expressed in the form of mean ± standard deviation ($x \pm s$) and the means were compared by the *t*-test. Statistical analysis was conducted by using the SPSS 13.0 software. The enumeration data was subject to the χ^2 test. $P < 0.05$ was considered to be statistically significant.

Results

1. Positive rates detected by RT-PCR of CK-7, CA125 and survivin in paraneoplastic tissues at different distances from orthotopic human epithelial ovarian carcinomas

Expression at different levels was observed in both the cancer tissue and the paraneoplastic tissues at different distances from the cancer. The positive rates decreased with the increase of the distance from the cancer and were relatively low in the ovarian tissue of normal nude mice Table 2.

2. Semi-quantitative expression levels detected by RT-PCR of CK-7, CA125 and survivin in paraneoplastic tissues at different distances from orthotopic human epithelial ovarian carcinomas (Table 3, Figure 1-3).

3. Positive rates detected by IHC of CK-7, CA125 and survivin in paraneoplastic tissues at different distances from orthotopic human epithelial ovarian carcinomas.

Table 2: Positive rates detected by RT-PCR of molecular markers in cancer tissue and paraneoplastic tissues at different distances (cases, %).

Tissue	Cases	CK-7	CA125	survivin
Cancer tissue	18	17(94.4)	17(94.4)	16(88.9)
Paraneoplastic tissues:				
1 mm - 1.5 mm from the cancer	18	13(72.2)[a]	14(77.8)[a]	14(77.8)[a]
1.5 mm - 3 mm from the cancer	18	6(33.3)[b]	7(38.9)[b]	5(27.8)[b]
3 mm - 4.5 mm from the cancer	18	4(22.2)[c]	5(27.8)[c]	4(22.2)[c]
Ovarian tissue of normal nude mice	20	3(15.0)	4(20.0)	3(15.0)

Compared with cancer tissue, a:$\chi2$=3.39, 3.25, 3.49, P>0.05; b:$\chi2$=5.72, 5.64, 5.88, P<0.05; c:$\chi2$=7.47, 7.26, 7.32, P<0.05.
Compared with normal ovarian tissue, a:$\chi2$=7.85, 7.81, 7.92, P<0.01; b:$\chi2$=3.59, 3.51, 3.37, P>0.05; c:$\chi2$=2.67, 2.92, 2.70, P>0.05.

Table 3: Expression levels detected by RT-PCR of molecular markers in cancer tissue and paraneoplastic tissues at different distances ($x \pm s$).

Tissue	Cases	CK-7	CA125	survivin
Cancer tissue	18	0.94±0.27	0.92±0.26	0.89±0.22
Paraneoplastic tissues:				
1 mm - 1.5 mm from the cancer	18	0.83±0.24[a]	0.86±0.26[a]	0.80±0.21[a]
1.5 mm – 3 mm from the cancer	18	0.36±0.12[b]	0.41±0.13[b]	0.32±0.10[b]
3 mm - 4.5 mm from the cancer	18	0.28±0.07[c]	0.33±0.09[c]	0.29±0.08[c]
Ovarian tissue of normal nude mice	20	0.19±0.05	0.21±0.06	0.20±0.05

Compared with cancer tissue, a:t=1.57, 1.49, 1.62, P>0.05; b:t=2.49, 2.37, 2.61, P<0.05; c:t=3.86, 3.25, 3.69, P<0.01.
Compared with normal ovarian tissue, a:t=3.97, 3.83, 3.66, P<0.01; b:t=1.62,1.58,1.51, P>0.05; c:t=1.94,1.87,1.79, P>0.05.

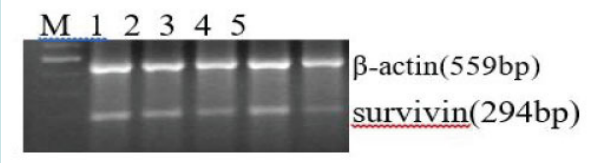

Figure 1: Semi-quantitative RT-PCR electrophoresis result of surviving M: Marker; 1-3) Paraneoplastic tissues successively 1 mm, 1.5 mm, and 3 mm from the cancer; 4) Cancer tissue; 5) Ovarian tissue of normal nude mice.

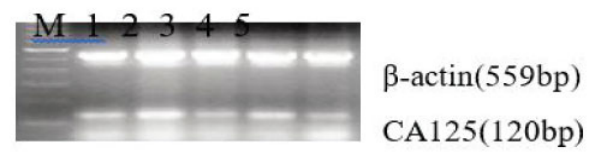

Figure 2: Semi-quantitative RT-PCR electrophoresis result of CA125 M: Marker; 1) Paraneoplastic tissue 1 mm from the cancer; 2) Cancer tissue; 3) Paraneoplastic tissue 3 mm from the cancer; 4) Paraneoplastic tissue 1.5 mm from the cancer; 5) Ovarian tissue of normal nude mice.

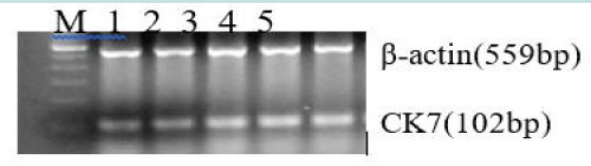

Figure 3: Semi-quantitative RT-PCR electrophoresis result of CK7 M: Marker; 1): Ovarian tissue of normal nude mice; 2-4) Paraneoplastic tissues successively 3 mm, 1.5 mm, and 1 mm from the cancer; 5) Cancer tissue.

Expression at different levels was observed in both the cancer tissue and the paraneoplastic tissues at different distances from the cancer. The positive rates decreased with the increase of the distance from the cancer and were relatively low in the ovarian tissue of normal nude mice (Table 4, Figure 4-9).

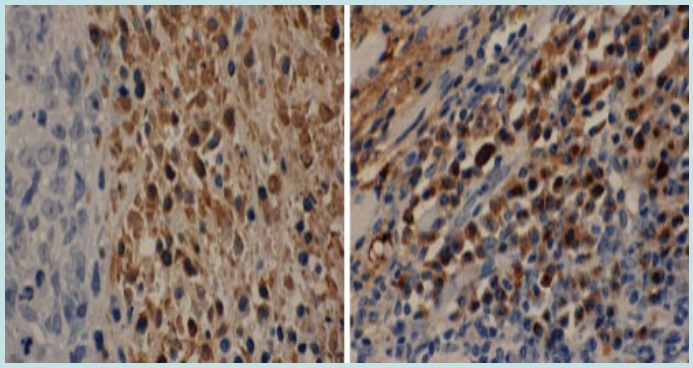

Figure 4: Expression of survivin (Two-step IHC ×200): cancer tissue and paraneoplastic tissue 1 mm-1.5 mm from the cancer Nucleus or cytoplasm appears to be brownish yellow granules.

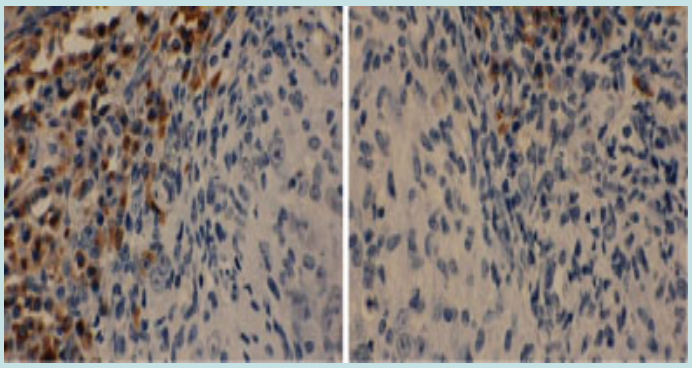

Figure 5: Expression of survivin (Two-step IHC ×200): paraneoplastic tissues 1.5 mm - 3 mm and 3 mm - 4.5 mm from the cancer Nucleus or cytoplasm appears to be brownish yellow granules.

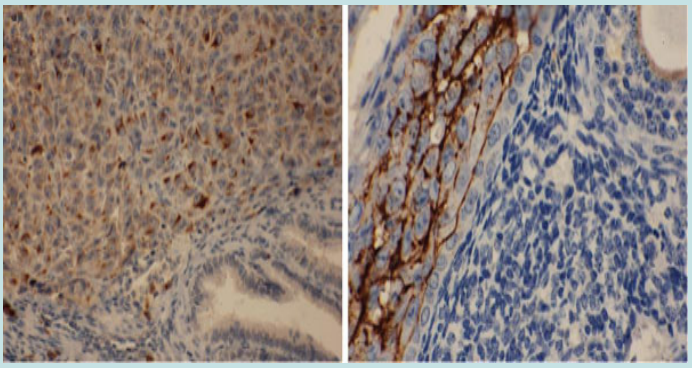

Figure 6: Expression of CK7 (Two-step IHC ×200): cancer tissue and paraneoplastic tissue 1 mm - 1.5 mm from the cancer cytoplasm appears to be brownish yellow granules.

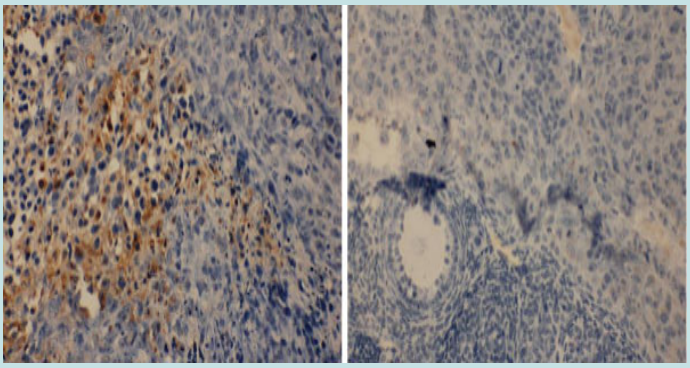

Figure 7: Expression of CK7 (Two-step IHC ×200): paraneoplastic tissues 1.5 mm – 3 mm and 3 mm - 4.5 mm from the cancer Cytoplasm appears to be brownish yellow granules.

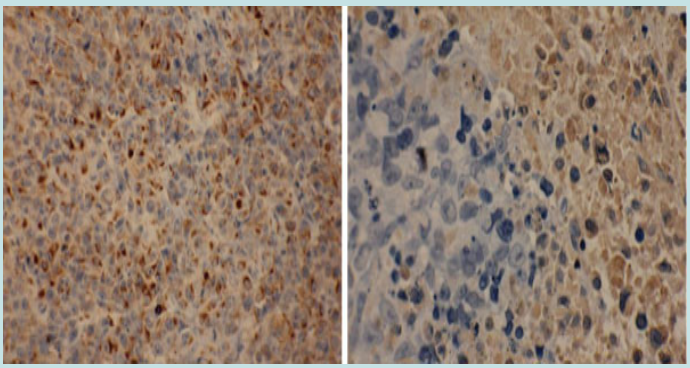

Figure 8: Expression of CA125 (Two-step IHC ×200): cancer tissue and paraneoplastic tissue 1 mm -1.5 mm from the cancer Cytoplasm or cell membrane appears to be brownish yellow granules.

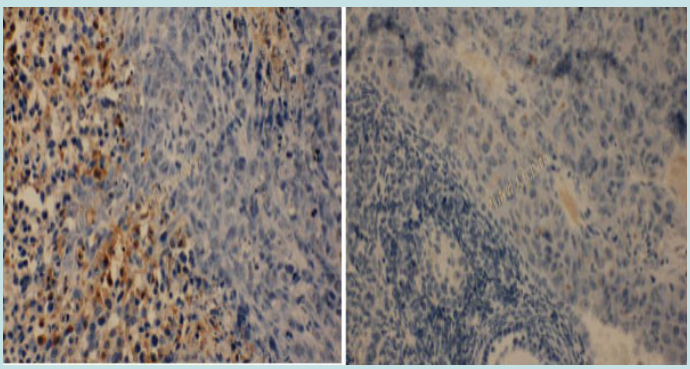

Figure 9: Expression of CA125 (Two-step IHC ×200): paraneoplastic tissues 1.5 mm – 3 mm and 3 mm - 4.5 mm from the cancer cytoplasm or cell membrane appears to be brownish yellow granules.

Table 4: Positive rates detected by IHC of molecular markers in cancer tissue and paraneoplastic tissues at different distances (cases, %).

Tissue	Cases	CK-7	CA125	survivin
Cancer tissue	18	17(94.4)	16(88.9)	17(94.4)
Paraneoplastic tissues:				
1 mm - 1.5 mm from the cancer	18	13(72.2)[a]	13(72.2)[a]	14(77.8)[a]
1.5 mm – 3 mm from the cancer	18	6(33.3)[b]	6(33.3)[b]	7(38.9)[b]
3 mm - 4.5 mm from the cancer	18	2(11.1)[c]	3(16.7)[c]	2(11.1)[c]
Ovarian tissue of normal nude mice	20	3(15.0)	2(10.0)	2(10.0)

Compared with cancer tissue, a:χ^2=3.39, 3.14, 3.48, P>0.05; b:χ^2=5.72, 5.61, 5.68, P<0.05; c:χ^2=7.63, 6.78, 7.63, P<0.01.

Compared with normal ovarian tissue, a:χ^2=7.42, 7.55, 7.56, P<0.01; b:χ^2=3.59, 3.46, 3.57, P>0.05; c: χ^2=2.56, 2.62, 2.39, P>0.05.

Discussion

When performing fertility-sparing surgeries for epithelial ovarian cancer, especially enucleation and ovary-sparing local tumorectomy, it is crucial to ensure complete removal of residual cancer cells or potential malignant cells. That's because there may be residual cancer cells or unstable potential malignant cells in the tissues surrounding a malignant tumor even if routine pathologic examination indicates normal results [10]. Further development of these cells may lead to tumors and this is the main reason why fertility-sparing surgeries for epithelial ovarian cancer fail to be widely applied in clinical practice.

The purpose of fertility-sparing surgeries for epithelial ovarian cancer is to furthest remove tumor tissues including microscopic lesions and potentially metastatic tumor cells. Theoretically, the farther the resection margin is away from the tumor, the safer the surgery will be. However, the volume of the ovary is small and larger distance between the margin and the tumor means more ovarian tissue would be cut off, thus making the conservative surgery lose its significance. The study on fertility preservation in women with borderline ovarian tumors has suggested that margins relatively close to the tumor tissue may lead to relatively high recurrence rate even if the pathological examination shows normal results [11]. That's because, although there are no tumor cells identifiable in routine pathological examination, some cells' biological behaviors have changed at the molecular level. Therefore, the truly safe surgical margin should cover the cells that are considered to be normal by routine pathological examination, but have changed at the molecular level, so as to achieve the goal of retaining normal ovarian tissue as much as possible and completely removing tumor tissues and potential lesions to lower down the recurrence rate.

When performing a conservative surgery, it is impossible to change the surgical margin too much to find the safe area for resection and the minimum safe margin must be determined quickly and accurately. No specific method used to screen for residual cancer cells has been developed. In recent years, a lot of studies have proved that IHC and RT-PCR are effective methods for screening residual cancer lesions, micrometastasis and occult metastasis [12,13]. Many studies have been carried out on the selection of molecular markers for the screening. Some researchers reported that gene expression of CD_{30}, T-cell receptor and BCR-ABl can be used to screen for residual microscopic lesions in transplanted ovarian tissues of patients with Hodgkin's disease,

lymphoma and leukemia respectively [14] and plays a good monitoring role. Inspired by this study, we determined to choose from the relevant genes that mediate ovarian cancer invasion and metastasis. According to the literature, CK-7 has a stable expression in primary ovarian cancer or ovary-derived metastases and is a commonly used indicator to help determine whether a metastasis is derived from the ovary [15]. CA125 is a commonly used tumor marker for epithelial ovarian cancer and has a high expression level in ovarian cancer tissues. CA125 is often used to determine whether there are residual lesions after chemotherapy for ovarian cancer and to further detect tissues that are considered to be negative by routine pathological examination [16]. The survivin gene has no or low expression in normal tissues and is a common molecular marker for monitoring tumor metastasis and estimating efficacy and prognosis [17]. We have carried out a series of studies on the expression of CK-7, CA125 and survivin in tissues adjacent to epithelial ovarian cancer and concluded that negative expression of CK-7, CA125 and survivin can be used as a criterion to exclude residual cancer and occult cancer [8]. In addition, we have preliminarily tested the tissues adjacent to human ovarian cancer and found that tissues over 1 cm from the cancer are close to the normal ovarian tissue in the expression levels. Thus, 1 cm can be regarded as the minimum safe distance for the removal of human ovarian solid tumors. This systematic study took nude mice as its research object, so the surgical margin in nude mice must be determined. The results of the study on human ovarian cancer are not applicable to the ovary of nude mice due to its small size. We used the above markers to screen for residual cancer cells in paraneoplastic tissues at different distances from orthotopic human epithelial ovarian carcinomas in nude mice and the results showed that the expression levels of CK-7, CA125 and survivin showed an obvious decreasing trend as the distance from the cancer increasing from 1 mm, to 1.5 mm and to 3 mm. The expression levels of the molecular markers in the paraneoplastic tissue within 1mm from the cancer were close to those in the cancer tissue and the expression levels in the paraneoplastic tissue over 3 mm from the cancer are close to those in the normal ovarian tissue. The results suggest that, although routine pathological biopsy of the paraneoplastic tissue within 1mm from the cancer indicated normal results, expression of CK-7, CA125 and survivin in the tissue changed significantly. The tissue may be an area with a high risk of small lesion residue or potential occult metastasis. The central area of a cancer lesion is characterized by much hypoxia or necrosis, so it is likely to be the ideal place to study tumor invasion and metastasis. On the other hand, molecular changes in the paraneoplastic tissue over 3 mm from the cancer are close to those in the normal ovarian tissue. Therefore, 3 mm is considered to be the minimum safe distance for removing orthotopic human epithelial ovarian carcinomas in nude mice and retaining normal ovarian tissue to the maximum extent, serving as a reference range for accurate selection of the surgical margin in further studies on fertility-sparing surgery. One of our previous studies came to the conclusion that positive rates of CK-7, CA125 and survivin expression are higher in paraneoplastic proximal tissues [18] and there is a certain recurrence rate after transplantation, so follow-up studies only use 3 mm as the safe distance of surgical margin and no longer compare with other distances.

Among the current methods for screening residual cancer cells, both IHC and RT-PCR techniques are highly specific and sensitive and commonly used in clinical research, but they also have some disadvantages. The main disadvantage of IHC is that the organic solvent treatment during slide preparation may easily cause partial loss of antigens in the tissue and thus affect the sensitivity of the results. In addition, certain subjectivity is involved in judging the results. RT-PCR technique has high sensitivity, but the amplification of contaminants may cause false-positive results. What's more, a lot of materials are needed during the test. Reimplantation of the normal paraneoplastic ovarian tissue, which we determined by using the two methods to detect the expression of CK-7, CA125 and survivin, did not cause cancerization, while reimplantation of the tissue with positive expression of the three markers led to significantly higher cancerization rate. This indicates that both the techniques are effective in screening residual cancer cells [8]. However, the screening methods should not be too complex. Thus, IHC may be more suitable for clinical application. The minimum safe margin determined by the above methods and the safety of the ovarian tissue thus retained still need to be further investigated.

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References

1. Jiang X, Yang J, Yu M, Xie W1, Cao D1, Wu M, et al. Oncofertility in patients with stage I epithelial ovarian cancer: fertility-sparing surgery in young women of reproductive age. World J Surg Oncol. 2017; 15 :154.
2. Hedbäck NE, Karlsen MA, Høgdall CK, Rosendahl M. Survival of selected patients with ovarian cancer treated with fertility-sparing surgery. Reprod Biomed Online. 2018; 37: 71-76.
3. Miller DM, Ehlen TG, Saleh EA. Successful term pregnancy following conservative debulking surgery for a stage IIIA serous low-malignant-potential tumor of the ovary: a case report. Gynecol Oncol. 1997; 66: 535-8.
4. Bergamini A, Petrone M, Rabaiotti E, Pella F, Cioffi R, Rossi EG, et al. Fertility sparing surgery in epithelial ovarian cancer in Italy: perceptions, practice, and main issues. Gynecol Endocrinol. 2018; 34: 305-308.
5. Melamed A, Rizzo AE, Nitecki R, Gockley AA, Bregar AJ, Schorge JO, et al. All-Cause Mortality After Fertility-Sparing Surgery for Stage I Epithelial Ovarian Cancer. Obstet Gynecol. 2017; 130: 71-79.
6. Gungorduk K, Asicioglu O, Braicu EI, Almuheimid J, Gokulu SG, Cetinkaya N, et al. The Impact of Surgical Staging on the Prognosis of Mucinous Borderline Tumors of the Ovaries: A Multicenter Study. Anticancer Res. 2017; 37: 5609-5616.
7. Moroney MR, Post MD, Berning AA, Sheeder J, Corr BR. An Evaluation of Frozen Section and Lymph Node Dissection Results for Mucinous Ovarian Tumors.Int J Gynecol Cancer. 2018; 28: 92-98.
8. Zhu GH, Wang ST, Yang ZX, Cai JH, Chen CY, Yao MZ, et al. Safety assessment of ovarian cryopreservation and transplantation in nude mice bearing human epithelial ovarian cancer. Asian Pac J Cancer Prev. 2012; 13: 4669-4675.
9. Casey L, Köbel M, Ganesan R, Tam S, Prasad R, Böhm S, et al. Comparison of p53 and WT1 immunohistochemical expression patterns in tubo-ovarian high-grade serous carcinoma before and after neoadjuvant chemotherapy. Histopathology. 2017; 71: 736-742.
10. Vidal F, Al Thani H, Haddad P, Luyckx M, Stoeckle E, Morice P, et al. Which Surgical Attitude to Choose in the Context of Non-Resectability of Ovarian Carcinomatosis: Beyond Gross Residual Disease Considerations. Ann Surg Oncol. 2016; 23: 434-442.

11. Helpman L, Yaniv A, Beiner ME, Aviel-Ronen S, Perri T, Ben-Baruch G, et al. Fertility preservation in women with borderline ovarian tumors - how does it impact disease outcome? A cohort study. Acta Obstet Gynecol Scand. 2017; 96: 1300-1306.
12. Grèze V, Kanold J, Chambon F, Halle P, Gremeau AS, Rives N, et al. RT-qPCR for PHOX2B mRNA is a highly specific and sensitive method to assess neuroblastoma minimal residual disease in testicular tissue. Oncol Lett. 2017; 14: 860-866.
13. Liu W, Qi L, Zu X, Li Y, He W, Tong S, et al. Preoperative marker panel for the prediction of residual tumor and the decision making for repeat transurethral resection. Urol Oncol. 2015; 33: 165.e9-14.
14. Shapira M, Raanani H, Cohen Y, Meirow D. Fertility preservation in young females with hematological malignancies. Acta Haematol. 2014; 132: 400-413.
15. Xing D, Jenson EG, Zwick CA, Rodriguez FJ, Kurman RJ. Atypical Proliferative (Borderline) Serous Tumor in the Brain: A Case Report. Int J Gynecol Pathol. 2018; 37: 52-56.
16. Nazmeen A, Maiti S, Mandal K, Roy SK, Ghosh TK, Sinha NK, et al. Better Predictive Value of Cancer Antigen125 (CA125) as Biomarker in Ovary and Breast Tumors and its Correlation with the Histopathological Type/Grade of the Disease. Med Chem. 2017; 13: 796-804.
17. Ju LL, Zhao CY, Ye KF, Yang H, Zhang J. Expression and clinical implication of Beclin1, HMGB1, p62, survivin, BRCA1 and ERCC1 in epithelial ovarian tumor tissues. Eur Rev Med Pharmacol Sci. 2016; 20: 1993-2003.
18. Zhu GH, Wang ST, Yao MZ, Cai JH, Chen CY, Yang ZX, et al. Screening of the residual normal ovarian tissue adjacent to orthotopic epithelial ovarian carcinomas in nude mice. Genet Mol Res. 2014; 13: 2978-2986.

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