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ORIGINAL ARTICLE

RCD24, B7-H4 and PCNA expression and clinical significance in ovarian cancer

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Summary

Purpose: *To analyze the correlations of cluster of differentiation 24 (CD24), B7-H4 and proliferating cell nuclear antigen (PCNA) with clinicopathological indexes of ovarian cancer by detecting the expressions of the three indexes, and further explore the clinical value of CD24, B7-H4 and PCNA detection in early diagnosis of ovarian cancer and screening of high-risk patients.*

Methods: *Gynecological paraffin-embedded blocks archived in the Department of Pathology of Suzhou Municipal Hospital from January 2015 to June 2017 were selected. There were 30 patients with benign epithelial ovarian tumor and 50 patients with malignant tumor according to the medical history and pathological data. In addition, 20 patients with normal ovarian tissues were enrolled as controls. Immunohistochemical streptavidin-peroxidase (SP) method was applied to detect CD24, B7-H4 and PCNA in different ovarian tissues, which were ultimately analyzed in combination with clinicopathological factors such as grade of differentiation and with or without lymph node metastasis.*

Results: *The expression levels of CD24, B7-H4 and PCNA in ovarian cancer tissues were remarkably higher than those in benign ovarian tumor tissues and normal ovarian tissues, and the expression levels of the three indexes in poorly- and moderately-differentiated ovarian cancer were notably higher than those in well-differentiated ovarian cancer ($p<0.05$). The expressions of CD24, B7-H4 and PCNA in ovarian cancer tissues with lymph node metastasis were significantly increased compared with those in ovarian cancer tissues without lymph node metastasis ($p<0.05$). The expressions of CD24, B7-H4 and PCNA in ovarian cancer tissues were positively correlated with each other ($p<0.05$).*

Conclusions: *The expressions of CD24, B7-H4 and PCNA have correlations with the occurrence, development, invasion and metastasis of ovarian cancer, and the combined detection may have clinical guiding significance for early diagnosis of ovarian cancer and screening of high-risk patients.*

Key words: *B7-H4, CD24, ovarian cancer tissues, PCNA*

Introduction

Ovarian cancer is one of the common tumors of the female genital organs, with an incidence rate right next to those of cervical cancer and endometrial cancer, as well as the highest death rate among the malignant gynecological tumors [1,2]. The occurrence, invasion and metastasis of malignant tumors have become the key of studies on tumors at present. The occurrence, development, invasion and metastasis of tumor is a continuous, complex and multi-step process [3-5]. Studies in recent years have manifested that cluster of differentiation 24 (CD24) (an adhesion molecule on the cell surface), co-stimulatory molecule B7-H4 and proliferating cell nuclear antigen (PCNA) play very important roles in the occurrence, development, invasion and metastasis of tumors [6-10]. Plenty of authors have investigated the detection of CD24, B7-H4 and PCNA alone, but there are few studies

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on the combined detection of these three indexes. This study aimed to examine the expressions of CD24, B7-H4 and PCNA in ovarian cancer tissues and normal ovarian tissues using immunohistochemical streptavidin-peroxidase (SP) method, clarify their expressions in different ovarian tissues and analyze their relationship with clinicopathological indexes of ovarian cancer, hoping to provide a basis for early diagnosis and treatment of this disease and guide clinical work in a better way.

Methods

Subjects

Archived paraffin-embedded blocks of inpatients in the Department of Gynecology of Suzhou Municipal Hospital from January 2015 to June 2017 were selected. Thirty patients with benign epithelial ovarian tumors and 50 patients with malignant tumors were selected according to the medical history and pathological data. Among them, there were 15 cases of well-differentiated tumor, 21 cases of moderately-differentiated tumor, 14 cases of poorly-differentiated tumor, 16 cases with lymph node metastasis and 34 cases without lymph node metastasis. In addition, 20 patients subjected to ovariectomy due to suspected ovarian cancer, but with normal ovarian tissues verified through pathology were enrolled as controls. No patients underwent radiotherapy or chemotherapy or took any hormone drug before the operation. Sections of all the cases were definitely diagnosed and reviewed by two senior pathologists in strict accordance with the World Health Organization (WHO) criteria. Written informed consent was obtained from all participants before study entry. This study was approved by the Ethics Committee of Suzhou Municipal Hospital.

Methods

The following consecutive steps were performed in this study:

a. Conventional deparaffinization and washing.
b. Antigen retrieval: The specimens were placed in citric acid buffer solution with a hydrogen-ion concentration (pH) of 7.4 at 100°C for antigen retrieval, which was then naturally cooled down to room temperature. Next, the specimens were washed twice with distilled water, followed by washing with 0.1 mmol/L phosphate-buffered saline (PBS) (pH=7.4) for three times (5 min per time).
c. Elimination of endogenous peroxidase activity: The specimens were placed in 3% H_2O_2 for incubation at room temperature for 10-15 min, so as to eliminate the activity of endogenous peroxidase, followed by washing with PBS for three times (5 min per time).
d. Addition of primary antibody in drops: The 0.1 mmol/L PBS was utilized to dilute the stock solution, of which CD24 and B7-H4 were diluted at 1:400, and PCNA was diluted at 1:200. The known positive specimens were taken as positive controls, and 0.1 mmol/L PBS served as the negative control in place of primary antibody.
e. Addition of biotin-labeled secondary antibody: Glass slides were washed with PBS for three times (5 min per time). Then, a proper amount of secondary antibody [1% bovine serum albumin (BSA)-PBS diluted at 1:300] was added in drops for incubation at room temperature for 30 min. The glass slides were washed again with PBS for three times (5 min per time).
f. Addition of horseradish peroxidase-labeled avidin (dilution and usage were the same as those of the secondary antibody).
g. Color development using diaminobenzidine (DAB) developer and counterstaining.
h. Dehydration, drying and sealing of sections.
i. Observation of films under a microscope: It was observed under the microscope that the positive signals of CD24 and B7-H4 were presented as yellowish-brown or sepia staining in the cell membrane and cytoplasm. Ten fields of vision at 400× magnification were observed in each section, which were judged by combining with the staining intensity and proportion of positive cells. The staining intensity scoring of the specimens was determined by referenced to the scoring methods in the literature.

Statistics

All statistics were performed using SPSS 19.0 software package (IBM, Armonk, NY, USA). Numerical data were presented as percentages, and chi-square test was used for the comparison between groups. Spearman's correlation test was used for the correlation analysis between indexes. $P<0.05$ suggested that the difference was statistically significant.

Results

Expressions of CD24 in different ovarian tissues

The positive expression rate of CD24 in ovarian cancer was 76.0%, which was increased remarkably compared with those in normal tissues and benign ovarian tumor tissues ($p<0.05$, Table 1). There was a statistically significant difference in the positive expression rate of CD24 between well-differentiated ovarian cancer tissues and poorly- and moderately-differentiated ovarian cancer tissues (26.67 vs. 88.57%, $p<0.05$). CD24 expression in ovarian cancer was associated with the grade of tumor differentiation, and the lower the grade of tumor differentiation, the higher the positive expression rate of CD24. Compared with that in the ovarian cancer tissues without lymph node metastasis, the positive expression rate of CD24 in the ovarian cancer tissues with lymph node metastasis was markedly elevated (87.50 vs. 38.24%, $p<0.05$), indicating that CD24 plays a vital role in the distant metastasis of ovarian cancer (Table 2).

Expressions of B7-H4 in different ovarian tissues

The ovarian cancer tissues had a notably higher positive expression rate of B7-H4 (80%) than normal ovarian tissues (0%) and benign ovarian tumor tissues (20%) (p<0.05, Table 1). The difference in the positive expression rate of B7-H4 between well-differentiated ovarian cancer tissues and poorly- and moderately-differentiated ovarian cancer tissues was statistically significant (40 vs. 85.71%, p<0.05). This implied that the lower the grde of tumor differentiation, the higher the positive expression rate of B7-H4. There was a statistically significant difference in the positive expression rate of B7-H4 between ovarian cancer tissues with and without lymph node metastasis (87.5 vs. 41.18%, p<0.05), indicating that B7-H4 is also involved in the distant metastasis of ovarian cancer (Table 2).

Expressions of PCNA in different ovarian tissues

In comparison with those in normal tissues and benign ovarian tumor tissues, the positive expression rate of PCNA was relatively higher in ovarian cancer tissues (78%, 0% and 23.33%, respectively) (p<0.05, Table 1). There was a statistically significant difference in the positive expression rate of PCNA among ovarian cancer tissues with different grades of differentiation (88.57 vs. 33.33%, p<0.05), implying that the PCNA expression in ovarian cancer is correlated with the grade of tumor differentiation. Compared with that in the ovarian cancer tissues without lymph node metastasis, the positive expression rate of PCNA in the ovarian cancer tissues with lymph node metastasis was remarkably increased (81.25 vs. 44.12%, p<0.05), suggesting that PCNA plays a role in the distant metastasis of ovarian cancer (Table 2).

Table 1. Expression of CD24, B7-H4 and PCNA in different ovarian tissue

		Normal ovarian tissue	*Benign ovarian tissue*	*Ovarian cancer tissue*	*p*
CD24	+	0	8	38	<0.05
	-	20	22	12	
	Positive rate	0%	26.67%	76%	
B7-H4	+	0	6	40	<0.05
	-	20	24	10	
	Positive rate	0%	20%	80%	
PCNA	+	0	7	39	<0.05
	-	20	23	11	
	Positive rate	0%	23.33%	78%	

Table 2. Relationship of CD24, B7-H4 and PCNA expression with pathology in ovarian cancer tissue

Characteristics	*n*	*CD24(+)*	*B7-H4(+)*	*PCNA(+)*
Differentiation grade				
Well-differentiated	15	4	6	5
Poorly- and moderately-differentiated	35	31	30	31
p		<0.05	<0.05	<0.05
Lymph node metastasis				
Yes	16	14	14	13
No	34	13	14	15
p		<0.05	<0.05	<0.05

Table 3. The correlation of CD24 and B7-H4 in ovarian cancer tissue

CD24 expression	*B7-H4 expression*			*r*	*p*
	+	-	*Total*		
+	35	3	38	0.592	<0.05
-	5	7	12		
Total	40	10	50		

Table 4. The correlation of CD24 and PCNA in ovarian cancer tissue

CD24 expression	*PCNA expression*			*r*	*p*
	+	-	*Total*		
+	34	4	38	0.576	<0.05
-	5	7	12		
Total	39	11	50		

Table 5. The correlation of B7-H4 and PCNA in ovarian cancer tissue

B7-H4 expression	*PCNA expression*			*r*	*p*
	+	-	*Total*		
+	35	5	40	0.518	<0.05
-	4	6	10		
Total	39	11	50		

Correlations among CD24, B7-H4 and PCNA expressions in ovarian cancer tissues

Among the 52 cases of ovarian cancer tissues, the Spearman's correlation analysis revealed that the positive expression of CD24 had significantly positive correlations with those of B7-H4 (r=0.592, p<0.05; Table 3) and PCNA (r=0.576, p<0.05; Table 4), and the positive expression of B7-H4 was significantly positively correlated with that of PCNA (r=0.518, p<0.05; Table 5).

Discussion

In recent years, the incidence rate of ovarian cancer is on the rise, and the patients with this disease present gradually in younger ages, with a mortality rate ranking first among the gynecologic tumors [1]. The prognosis and survival rate of patients can be improved significantly through early diagnosis and active treatment of ovarian cancer, so searching for efficacious and accurate diagnostic methods currently becomes the key to research on ovarian cancer. With the proposed theory of tumor stem cells in the past few years, multiple markers on the surface of the tumor stem cells have been discovered. In addition, studies on their mechanisms have manifested that those cells exert crucial effects on the occurrence, development, relapse and drug resistance of tumors, so they are gradually applied in the early diagnosis and targeted therapy of ovarian cancer [3-5]. In this study, immunohistochemistry was utilized to detect the expressions of CD24, B7-H4 and PCNA in different ovarian tissues, as well as their correlations. It is now discussed as follows:

CD24, as a type of highly glycosylated protein with a low molecular weight, plays an important role in regulating the growth, proliferation and migration of tumor cells as an adhesion molecule. Besides, its high expression and expression pattern are closely related to the survival and prognosis of the patients [6-8]. Therefore, it is a valuable indicator for the diagnosis and prognosis of a variety of tumors. Previous studies have discovered that CD24 is rarely expressed in normal ovarian tissues, but it is highly expressed in ovarian cancer tissues. Moreover, patients with CD24 overexpression have higher grade malignancy and poorer prognosis. According to this study, the positive expression rate of CD24 in malignant ovarian tumor tissues was obviously higher than that in benign ovarian tumor tissues, and differences of the positive expression rate existed in different ovarian tissues. Furthermore, it implies that CD24 is closely associated with the grade of differentiation and metastasis of the tumor. The higher the malignancy and the staging, the lower the grade of differentiation and the higher the CD24 expression intensity.

B7-H4 has a negative regulatory effect on the T cell-mediated immunity, which is mainly manifested as inhibition of T cell proliferation, reduction of interleukin-2 (IL-2) production and control of cell cycle progression in G0/G1 phase, thus playing a vital part in the process of tumor escape [9,11]. B7-H4 is extensively expressed in plenty of tumor tissues, but there is limited expression of B7-H4 protein. In this experiment, the positive expression rate of B7-H4 varied among different ovarian cancer tissues. Meanwhile, the B7-H4 expression in ovarian cancer tissues was associated with the grade of tumor differentiation and lymph node metastasis. Therefore, it is conjectured that the B7-H4 expression may promote the occurrence and metastasis of ovarian cancer to some extent.

PCNA is an accessory protein of DNA polymerase D, which participates in the replication and repair of DNA strands and can be regarded as a specific marker for the S phase of the cell cycle. Its expression level can reflect the degree of cell proliferation, thus serving as a commonly used indicator for judging cell proliferation activity [10]. Some studies have pointed out that PCNA has correlations with the differentiation, infiltration and relapse of tumor as well as the metastasis of lymph node or organ [12,13]. The results of these studies indicated that ovarian cancer tissues with a lower degree of differentiation and metastasis have higher malignant potential more active cell proliferation and higher positive expression rate of PCNA.

In this study, the correlation analysis on the CD24, B7-H4 and PCNA expressions in ovarian cancer tissues correvealed that they were positively related to each other, and the combined detection had reference values for the diagnosis and treatment of ovarian cancer.

In conclusion, research on the expressions of CD24, B7-H4 and PCNA in different ovarian tissues in this study revealed that their positive expressions had statistical significance in malignant ovarian tumor. CD24, B7-H4 and PCNA are correlated to each other, and the combined detection can increase the accuracy of diagnosis. In addition, all the three indexes had important reference values and clinical guiding significance to the screening of high-risk patients, clinical staging and metastasis of ovarian cancer.

Conclusions

The positive expressions of CD24, B7-H4 and PCNA had associations with the occurrence and development of ovarian cancer, and their combined detection is of great clinical value for the diagnosis and treatment of ovarian cancer and screening of high-risk patients.

Conflict of interests

The authors declare no conflict of interests.

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