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Expression of PKCα, PKCε, and P-gp in epithelial ovarian carcinoma and the clinical significance

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Summary

Aim: To inspect the expression of two protein kinase PKC isozyme hypotype PKCα and PKCε in the epithelial ovarian carcinoma tissue, and investigate their relation with multi-drug resistance with P-glycoprotein (P-gp) medium. *Materials and Methods:* Adopted immunohistochemistry SP method to determine expression of PKCα, PKCε, and P-gp in 64 cases of epithelial ovarian carcinoma, 18 cases of epithelial borderline ovarian carcinoma, 15 cases of epithelial ovarian benign tumor, and 15 cases of normal ovarian tissue. *Results:* The expression of PKCα, PKCε, and P-gp in the epithelial ovarian carcinoma is obviously higher than expression in the normal, benign. and borderline epithelial ovarian carcinoma; the expression of PKCα, PKCε, and P-gp in the recurrent carcinoma tissue is obviously higher than that in the person with initial treatment; the expression of above-mentioned three indicators in epithelial ovarian carcinoma is unrelated with the pathological type, pathological grade, and clinical stage during initial treatment of the carcinoma; there is a close relation among PKCα, PKCε, and P-gp in epithelial ovarian carcinoma ($p < 0.01$). It is indicated through research that PKCα, PKCε, and P-gp is related with the survival time and poor prognosis of the patient of epithelial ovarian carcinoma, i.e., the positive expression rate of PKCα, PKCε, and P-gp of the person with recurrent carcinoma is higher than that of the person without recurrent carcinoma ($p < 0.05$). However, the survival rate of the patients with positive expression of three indicators is remarkably lower than those with negative expression ($p < 0.05$). *Conclusion:* There is a consistency between expression of PKCα, PKCε, and P-gp in the epithelial ovarian carcinoma, which indicates that the expression of both plays an important role in generation of drug resistance in chemotherapy of ovarian carcinoma with P-gp medium. Joint detection of three indicators has an active guiding role in judgment of the therapeutic effect of clinical chemotherapy and prognosis estimation of the patient.

Key words: Epithelial ovarian carcinoma; PKCα;PKCε; P-gp; Immunohistochemistry; Multi-drug resistance.

Introduction

Ovarian carcinoma is one of three major malignancies of female, the five-year survival rate is low, and chemotherapy is the main adjuvant therapy means. However, later clinical stage at definite diagnosis and chemotherapy resistance are the main reasons for poor prognosis and short survival time of the patients. It is indicated through research that the chemotherapy resistance mechanism of the tumor cells is quite complicated. The overexpression of P-glycoprotein (P-gp) coded by the multi-drug resistance gene is the most important. It is indicated through recent research that there is a certain relation and correlation between protein kinase C (PKC) and P-gp in tumor chemotherapy resistance mechanism and expression of multi-drug resistance gene. In the research, the immunohistochemistry method is adopted to determine the expression situation of PKCα, PKCε, and P-gp in benign and malignant tumors and normal tissues, and it is found that PKCα and PKCε can participate in formation of ovarian carcinoma multidrug resistance (MDR) through adjustment of P-gp expression, which provides the theoretical basis for reversion of ovarian carcinoma MDR and judgment of chemotherapy effect and prognosis.

Materials and Methods

Patients and specimens

The specimens were randomly selected from the Pathology Department of the First Affiliated Hospital of Henan University of Science and Technology, Lou Yang, China, including 112 cases of paraffin embedding filed from 2005 to 2012 and 64 cases of ovarian carcinoma. They included 41 cases of tissue specimens through initial treatment operation without chemotherapy and 23 cases of tissue specimen through secondary cytoreductive surgery with tumor recurrence after chemotherapy; 28 cases with serous cystadenocarcinoma, 13 cases with mucinous cystadenocarcinoma, 19 cases with endometrioid carcinoma, and four cases of adenocarcinoma; as for pathological grade, 12 cases were Grade I, 27 cases Grade II, and 25 cases Grade III; according to FIGO staging standard, eight cases of Stage I, seven cases of Stage II, 41 cases of Stage III, and eight cases of Stage IV; 41 cases of patients underwent four to ten chemotherapy treatment courses after initial operation treatment of ovarian carcinoma, including 30 cases with PAC (cisplatin, adriamycin, cyclophosphamide) scheme and 11 cases with TP (cisplatin, palclitaxel) scheme. All 23 cases of recurrent ovarian carcinoma were within six months from discontinuation of chemotherapy [1]. Fifteen cases were benign epithelial ovarian carcinoma, 18 cases of epithelial ovarian borderline carcinoma, and 15 cases of normal ovarian tissue. The ages of all cases were from 74 to 28 years, with the average age of 59 years.

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The three indicators include P-gp membrane expression and PKCα and PKCε cytoplasm expression (×200).

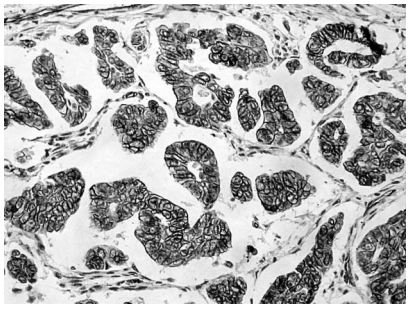

Figure 1. — P-gp (+++) ovarian endometrioid carcinoma.

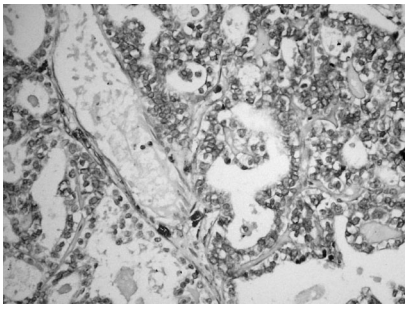

Figure 2. — PKCε (+++) ovarian serous cystadenocarcinoma.

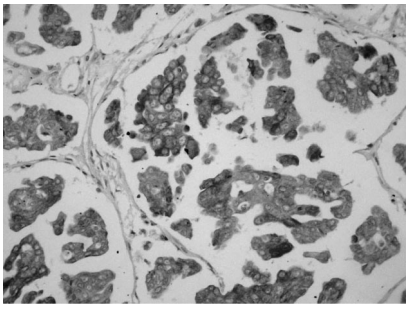

Figure 3. — PKCα (+++) ovarian serous cystadenocarcinoma.

Table 1. — *Expression of P-gp, PKCα, and PKCε in initial treatment and recurrent ovarian carcinoma tissue.*

Item	Number of cases	P-gp		PKC_α		PKC_ε	
		Positive	%	Positive	%	Positive	%
Initial treatment	41	15	36.6	18	43.9	25	61.0
Recurrent	23	20	87.0	19	82.6	21	91.3
X^2		15.087		9.051		6.705	
p		<0.01		<0.01		<0.05	

Immunohistochemistry

All specimens were fixed in 10% formaldehyde solution and underwent conventional paraffin embedding. Serial four sections were placed on glass slides and pretreated with TEN g/l polyphosphate lysine. Antigen retrieval was performed by microwaving sections in 0.01 mol/l citrate buffer for ten minutes and then cooled to room temperature. Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide in methanol for 20 minutes at room temperature and non-specific binding was blocked by incubation with 5% bovine serum albumin in phosphate-buffered saline(PBS) at room temperature. After PBS washing for three times, the specimens were reacted overnight at 4°C with P-gp or PKC_ε monoclonal antibody.The main reaction procedures of immunohistochemistry were conducted in accordance with instructions of the test kit. In each test, the known positive film was the positive control and PBS was the negative control instead of the primary antibody.

Result judgment

Five high power fields were randomly selected and counted over 500 tumor cells. PKCα and PKCε was judged as the positive cells with the membrane or cytoplasm color and P-gp was judged as positive cells with the membrane color. The semi-quantitative was conducted in accordance with the number of positive cells and cell staining intensity [2]: no positive cell: 0 points, positive cells: 1% ~30%: 1 point, 31% ~70% : 2 points, 71%~100% : 3 points; 0, 1, 2, and 3 points were counted in accordance with the positive staining intensity (no color: 0 points, light brown: 1 point, brown yellow: 2 points, and dark brown: 3 points). Two scores of each section were accumulated. 0 points: negative, 1~2 points: weakly positive (+), 3~4 points: positive (++), and 5~6 points: strongly positive (+++). The negative in the experiment referred to weakly positive to strongly positive (+ ~ +++).

Results

Expression of PKCα, PKCε, and P-gp in various ovarian tissues and their relation with parameters of clinical pathology

The positive staining of PKCα, PKCε, and P-gp in ovarian carcinoma is brown, and is positioned in the cell membrane or cytoplasm (Figures 1-3). The PKCα positive rates

in the borderline and malignant ovarian tissue were 6% and 64%, respectively, and there was no expression in the normal and benign ovarian tissues (X^2= 45.383, $p < 0.01$). The expression rates of PKCε in benign, borderline, and malignant tissues were 7%, 11%, and 73%, respectively, and there was no expression in the normal tissue (X^2 = 53.364, $p < 0.01$). The expression rates of P-gp in borderline and malignant tissues were 17% and 55%, respectively, and there was no expression in the normal and benign ovarian tissues (X^2 = 28.777, $p < 0.01$). The above-mentioned three indicators included P-gp membrane expression and PKCα and PKCε cytoplasm expression. Through X^2 inspection, the discrepancy in the tissue type, pathological grade, and clinical stage of negative and positive groups of PKCα, PKCε, and P-gp in ovarian carcinoma was not significant ($p > 0.05$).

Expression of P-gp, PKCα, and PKCε in initial treatment and recurrent ovarian carcinoma tissue is shown in Table 1. It can be seen that the positive expression rate of above-mentioned three indicators in the patient with recurrent ovarian carcinoma tissue was obviously higher than that in the patient with initial treatment, which is of difference significance.

Table 2. — *Relation among PKCα, PKCε, and P-gp expression.*

P-gp	PKC_α		PKC_ε	
	Negative	Positive	Negative	Positive
Negative	18	8	19	11
Positive	7	31	6	28

Table 3. — *Expression of P-gp, PKCα, and PKCε and relation with survival rate with ovarian carcinoma.*

	P-gp		PKC_α		PKC_ε	
	Positive	Negative	Positive	Negative	Positive	Negative
Survival rate (%)	5.7	48.3	8.1	55.6	10.9	72.2
p	0.021		0.014		0.002	

Relation among PKC_α, PKC_ε, and P-gp expression

The Kappa internal consistency coefficient was used to inspect the relation among PKCα, PKCε, and P-gp expression and the results showed that there was consistency between expression of PKCα and P-gp, and Kappa coefficient was 0.511, $p < 0.01$. Similarly, there was good consistency between PKCε and P-gp expression, and Kappa coefficient was 0.461, $p < 0.01$ (Table 2).

Expression of P-gp, PKCα, and PKCε and relation with recurrence and prognosis

The follow-up survey conducted in 64 cases of epithelial ovarian carcinoma for an average 37.5 months (three to 72 months), with 23 cases of recurrence, included nine cases of P-gp (+) /PKC_α (+) /PKC_ε (+), five cases of P-gp (+) / PKC_α (-) / PKC_ε (+), seven cases of P-gp (+) / PKC_α (+) / PKC_ε (-), and two cases of P-gp (-) / PKC_α (+) / PKC_ε (+). The positive expression rate of P-gp, PKCα, and PKCε of the patient with recurrence was obviously higher than that of the patient without recurrence ($p < 0.05$). Kaplan-Meier method was adopted to analyze the survival time of 64 cases of ovarian carcinoma patients and expression of P-gp, PKCα, and PKCε, and the survival rate of the patients with positive expression of P-gp, PKCα, and PKCε was lower than those with negative expression ($p < 0.05$) (Table 3, Figures 4-6).

Discussion

Ovarian malignancy is one of three major malignancies of female genitals and the mortality ranks the first place among the gynecological malignancies. Epithelial ovarian carcinoma accounts for about 90%, and the principle treatment is surgical, aided with chemotherapy and radiotherapy. The chemical drug treatment is the main adjuvant therapy for ovarian carcinoma. Furthermore, it is found through research that compared with other solid carcinomas, the overall response rate to chemotherapy of epithelial ovarian carcinoma is up to 60% to

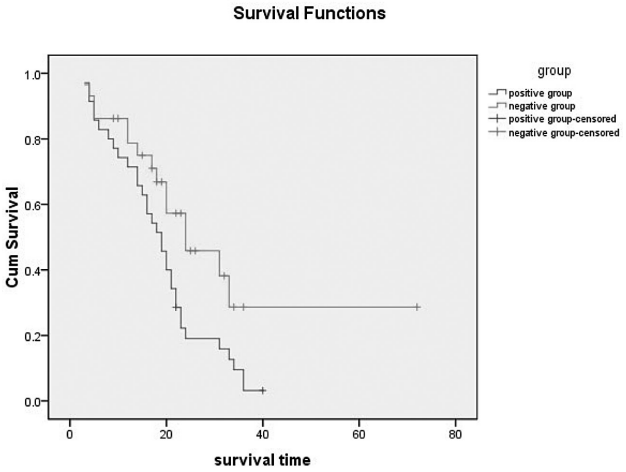

Figure 4. — Kaplan-Meier survival curve of patients with positive and negative P-gp expression.

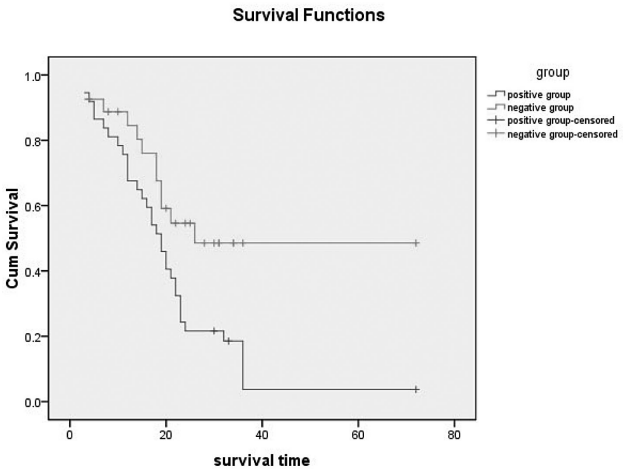

Figure 5. — Kaplan-Meier survival curve of patients with positive and negative PKC_α expression.

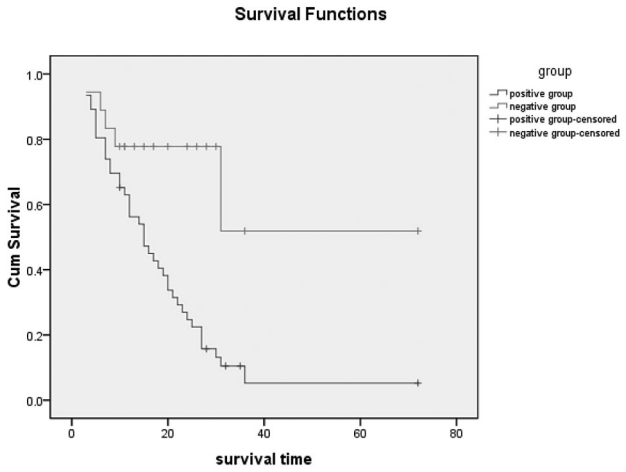

Figure 6. — Kaplan-Meier survival curve of patients with positive and negative PKC_ε expression.

70%. However, with increase of the treatment course of chemotherapy, at least 80% of chemotherapy patients have acquired MDR, causing failure of chemotherapy, which is the major barrier for a successful ovarian cancer therapy. P-gp,a transmembrane ATP-dependent efflux pump, is encoded by the mdr-1 gene, which can recognize endogenous metabolites and xenobiotics as substrates, including anticancer drugs such as anthracyclines, epipodophyllotoxins, and Vinca alkaloids. Moreover, the overexpression of P-gp in tumor cells is the main mechanism of multi-drug resistance [3]. It is found in the present experiment that there was no positive expression found in the normal ovarian and benign ovarian tumor, which further confirms that the ovary is not the enrichment organ of P-gp [4]. The expression of P-gp in ovarian carcinoma is higher than that in the normal ovarian tissue, benign, and borderline epithelial ovarian carcinoma tissue. Furthermore, the expression in the recurrent case of ovarian carcinoma within half a year after chemotherapy for numerous times was obviously higher than that in the patient with initial treatment, generating the acquired drug resistance. The result is consistent with the research at home and abroad. With the in-depth investigation of the chemotherapy resistance mechanism in recent years, P-gp has been recognized as the drug resistance index of malignancy. In research, P-gp is considered as the drug resistance index for ovarian carcinoma, to investigate the influence of PKC, on the ovarian carcinoma drug resistance, and possible approach.

PKC is a kind of widespread phospholipid-dependent enzyme, which can mediate the oncogene signal, participate in regulation of cell cycle and play a role in regulating cell growth and apoptosis. Therefore, it is related with occurrence and development of the tumor in multiple links. It is reported by Prevostel *et al.* [5] that the transposition of PKC in the cell can make PKC close to different protein substrates in the subcellular region, so that the cell will cause different external reactions. Therefore, PKC activity of multiple tumor tissue cells is high, and the hypotype expression is changed. It is prompted in the research that the expression of two hypotypes of PKCα and PKCε in ovarian carcinoma is higher than that in the normal tissue as well as benign and borderline epithelial ovarian carcinoma, which further reflects that PKC may participate in occurrence and development of ovarian carcinoma. PKC is closely interrelated to signal transduction of cell proliferation, differentiation, and apoptosis. On the one hand, the activated PKC can enable phosphorylation of some tumor proteins or enzymes in the cells, and the phosphorylated proteins enter the core to activate the specific gene, and increase transcription and expression; on the other hand, a portion of PKC hypotype or hydrolyzed PKC enters the core to directly combine DNA, which influences gene expression based on transcription, causing disorders in the cellular pathway. Therefore, PKC may participate in MDR formation through above-mentioned mechanism [6]. Zhan *et al.* [7] proposed that PKC directly influences expression of different drug resistance proteins and the phosphorylation degree. The overexpression of PKC or activity increase can enable phosphorylation of P-gp coded by MDR gene and generate MDR phenotype. Lee *et al.* [8] used a kind of inhibitor Go6976 of PKCα, which can reduce MDR expression, and increase doxorubicin-induced apoptosis. Bellrao *et al.* [9] used the inhibitor of PKC to act on the breast cancer cell which was resistant to doxorubicin. The result is that the inhibitor retards PKC activity, reduces P-gp phosphorylation, and increases intracellular drug accumulation. Therefore, the P-gp mediated MDR is blocked. On the other hand, it is reported that PKC activators were transfected with full-length PKC-alpha genes driven by the ecdysone promoter imported through transfection in MCF7 cells which enabled the stable expression of PKCα [10]. It is believed that PKCα may participate in P-gp transcription and regulate the expression. In addition, Sun *et al.* [11] found that QA3 was a derivative of the substituted 1,3-dimethy1-1H-quinoxalin-2-ones, which can suppress expression of P-gp in MDR cancer cells. Its mechanism is that QA3 significantly decreases the intracellular level of ATP, stimulates ATPase activity in membrane microsomes, and decreases PKC activity. High expression of PKC and P-gp in chemotherapy resistance of recurrent ovarian carcinoma was found in the present research, and there was a positive correlation between PKC and P-gp.

At present, it has been found that PKC has 12 hypotypes in mammalian cells. PKC hypotypes have the heterogeneity, and different extracellular stimuli will activate different PKC hypotypes, and the expressions of PKC hypotypes in various cells are different. In different PKC isozymes, PKCα is the main modifier of MDR phenotype. It is found in many drug resistant cell lines (for example, KB, MCF7, P388, rat 180 and UV - 2237M cells) that the rising level of PKCα is on average 30 times. It is displayed through the research that the expression of PKCα in the chemotherapy resistance recurrent carcinoma is significantly higher than that in the initial treatment, and there is a good consistency with P-gp, i.e. there is a close relation between two indicators, which indicates that PKCα may play an important role in acquired drug resistance of ovarian carcinoma. Its expression is obviously related with P-gp expression. This is consistent with the results of the research conducted by Wang *et al.* [12]. Beck *et al.*[13] analyzed the cancer cells of five cases of patients with ascites ovarian carcinoma, and found that the levels of MDRl and PKCε mRNA in four cases were relatively increased, one case was remarkably increased after chemotherapy, which may have been caused by PKCε that activated the drug resistance gene. Through research of prostate cancer cell line LNCa P, Flescher *et al.* [14] believe that PKCε can be the signal molecule for generation of P-gp. It is also prompted in the present research that there is a consistency between PKCε and P-gp. Therefore, it is indicated that PKCα and PKCε is related with ovarian carcinoma P-gp mediated MDR, and the function is exerted in two aspects of increasing P-gp phosphorylation and adjusting transcription.

During establishment of drug resistance cell line, the acquisition of MDR process is always related with MDRl gene amplification. However, with in-depth research of MDR mechanism of tumor patients, it is believed that the overexpression of P-gp in malignant tumor cells can be determined, but whether it is caused due to MDRl gene amplification cannot be determined. On the contrary, it is believed that a rise of MDRlmRNA and P-gp is activated by the external stimuli, therefore rising the MDRl gene. Therefore, it is inferred that blockage of MDRl gene activation mechanism can suppress MDR phenomenon in malignancy. Some scholars suppress the PKC signal pathway, to eliminate activation of MDR1 gene [15]. Therefore, the present authors believe that it is more effective to look for the signal pathway of MDR1 gene than direct use of reversal agent to suppress P-gp, if the acquired MDR of ovarian carcinoma is blocked in the upstream of P-gp. High expression of PKCα.PKCε and P-gp in chemotherapy resistance of recurrent ovarian carcinoma is found in the research, and there is a consistency between PKCα, PKCε, and P-gp. Therefore, in the future research, we can apply the antisense oligonucleotide, monoclonal antibody and transfected wild-type P^{53} of PKCα and PKCε in the molecule level to reverse the ovarian carcinoma MDR, and apply it in clinical trials. In addition, in recent years, with increase of cisplatin and doxorubicin resistance in the ovarian carcinoma chemotherapy drugs, paclitaxel is applied more widely as the first-line drug. However, scholars found through research that the drug resistance mechanism of Paclitaxel includes expression of P-gp, PKC expression change and so on [16]. The patients with ovarian carcinoma in this paper underwent TP or PAC treatment, which is the common first-line scheme for ovarian carcinoma. The high expression of PKCε and P-gp is also prompted in the generated chemotherapy resistance mechanism, which is consistent with the aforementioned research.

Through analysis with Kaplan-Meier survival curve, it was shown that the survival time of the patient with positive expression of PKCα, PKCε, and P-gp was obviously shorter than patient with negative expression of PKCα, PKCε, and P-gp. It is prompted that whether PKCα, PKCε, and P-gp are expressed may become the important indicator of tumor prognosis.

In summary, PKCα and PKCε expression is obviously related with ovarian carcinoma tissue chemotherapy resistance, which may play an important role in P-gp mediated ovarian carcinoma MDR. Therefore, in order to improve ovarian carcinoma chemotherapy effects, the combined drugs should be used, and the reversal agent in connection with various hypotypes of PKC can also be applied, which will remarkably improve the sensitivity of malignancy to chemotherapy. In addition, patients with ovarian carcinoma will be subject to determination of PKCα, PKCε, and P-gp, which is helpful for prediction of the chemotherapy results and prognosis judgment, and also helpful for the clinician to reasonably select the chemotherapy drugs based on the expression, with the goal to guide clinical treatment.

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