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Original Article

Value of serum p53, PKD1, and MAP2K4 in evaluating the condition and prognosis of endometrial carcinoma

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Abstract: Objective: To analyze the p53, PKD1, and MAP2K4 expressions in serum of patients with endometrial carcinoma (EC) and their prognostic value. Methods: A total of 84 patients with EC who were treated in our hospital between January 2018 and January 2020 were enrolled into a research group. There were 50 healthy individuals over the same time who were included in a control group for a retrospective analysis. qRT-PCR was used for quantifying the relative levels of p53, PKD1, and MAP2K4 in the serum of the control group and the research groups (in both cancer and paracancerous tissues). The associations of p53, PKD1, and MAP2K4 with pathological features of EC were analyzed. Patients were followed up for 1 year to observe their death and analyze the associations of p53, PKD1, and MAP2K4 with prognosis of EC. Results: The patients with EC had low p53 and MAP2K4 levels and high PKD1 levels ($P<0.05$). The p53, MAP2K4, and PKD1 levels in serum were relevant to EC differentiation, FIGO stage, lymph node metastasis, and deep myometrial invasion ($P<0.05$). During the follow-up of prognosis, the serum levels of p53 and MAP2K4 in dead patients were lower than those in surviving patients. PKD1 in former patients was higher ($P<0.05$). Conclusion: The low expressions of p53 and MAP2K4 and high expression of PKD1 in EC cases were related with disease progression. These expressions can help effectively evaluate the prognosis and survival of patients. They are of crucial research and reference significance for future diagnosis and therapy.

Keywords: p53, PKD1, MAP2K4, endometrial carcinoma, prognosis evaluation

Introduction

Endometrial carcinoma (EC) is a malignancy originating from endometrial epithelium. It is more frequently seen in perimenopausal and menopausal women. Its incidence is second only to cervical cancer among gynecological tumors, accounting for 20-30% of all gynecological malignancies [1]. A survey implies that 300,000 new cases suffered from EC worldwide in 2019. The incidence presented a trend of annual increase [2]. The specific pathogenesis of EC is under investigation. Clinically, endocrine disorders, obesity, reproductive diseases, estrogen, and lifestyle are deemed as factors that cause EC. A typical symptom is vaginal bleeding. At the initial stage, the amount of abnormal bleeding is usually small. EC is likely to be overlooked or mishandled by patients. The only clinical diagnostic method for EC is a pathological biopsy. This makes early screening difficult, contributing to the result that most EC cases have reached the middle and late stages at diagnosis [3]. For early EC, the ideal effect can usually be achieved by radical surgery or radical surgery combined with radiotherapy and chemotherapy. With the growth of the disease, EC developes lymphatic metastasis and hematogenous metastasis in different degrees. This greatly increases the difficulty of therapy, and makes the prognosis extremely pessimistic [4]. Statistics show that the survival rate of patients with advanced EC after therapy is only 10-18 months. The total mortality within 5 years is up to 50-70% [5]. Researchers have continued to explore novel ways for diagnosis and therapy of EC.

Continued research explores the pathogenic mechanism of tumors from the molecular point of view. Researchers found this to be a breakthrough to overcome diseases in the future [6]. Research by Hassan et al. [7] shows that a variety of genes and encoded proteins impact the occurrence of tumors through regulating the life cycle of cells and the activities of organs and

tissues. Currently, p53 gene is deemed a crucial tumor suppressor gene in human body. p53 has mutations in over 50% of tumors and abnormal p53 expression in EC cases [8]. During mutation, p53 binds to specific sites in the form of tetramer. This trans-activates the expression of downstream growth suppressor genes, participating in the development of tumors [9]. Among the downstream genes associated with p53, PKD1, and MAP2K4 are strongly bound up with reproductive system tumors and have important impacts on mediating cell-cell and cell-cytoplasm interactions [10, 11]. Recently, the abnormal expression of PKD1 and MAP2K4 in gynecological tumor diseases such as breast cancer and ovarian cancer has been confirmed, but their associations with EC are under investigation.

The molecular pathogenesis of tumor diseases cancompensate for the limitations of early tumor screening, improve the early detection rate of the disease, and lay the foundation for timely treatment of patients. Due to the lack of reliable disease assessment indicators for oncological diseases, there is a lack of clinical ssessment tools for patients' disease development. It is difficult to control their prognosis. Targeted therapy through a molecular perspective will likely achieve better results and safety than current conventional treatment modalities. With the increasingly high incidence of EC, this study quantified p53, PKD1, and MAP2K4 in EC cases to further understand the possible pathogenesis of EC and provide reliable reference for finding new diagnosis and therapy schemes of EC and its associated research.

Materials and methods

Patient information

A total of 84 EC patients admitted to our hospital between January 2018 and January 2020 were enrolled into a research group. There were 50 healthy individuals over the same time who were included in a control group for retrospective analysis [Ethics approval: 2017 (review) 071a]. All the subjects provided signed informed consent forms.

Inclusion and exclusion criteria

The inclusion criteria for the research group: (1) patients met the clinical manifestations of EC and confirmed to have it by pathology biopsy in our hospital; (2) patients who received surgery or surgery combined with radiotherapy and chemotherapy after admission to the hospital; (3) patients whose sections of cancer tissue and paracancerous tissue were obtained with the consent of the patients during the operation for follow-up detection; (4) patients >18 years old with detailed medical records. The exclusion criteria for the research group: (1) patients with other tumors, cardio-cerebrovascular diseases, autoimmune defects, infectious diseases, or mental diseases; (2) pregnant patients and lactating patients; (3) end-stage EC patients; (4) patients who had received surgery, radiotherapy, chemotherapy, or antibiotics within half a year before admission. The inclusion criteria of the control group: (1) patient >18 years old, with detailed medical records; (2) patients with normal physical examination results; (3) patients without a history of major diseases.

Sample collection

On admission, both groups collected fasting venous peripheral blood 4 mL in a coagulation-promoting tube, placed 30 min at room temperature, then centrifuged (1505×g, 4°C) 15 min to get serum, which was stored in a refrigerator at -80°C for further tests. With the consent of the patients, the remaining tissue from the pathological biopsy was cryopreserved for our follow-up study. The remaining cancer tissue and paracancerous tissue sections of the patients were obtained for the present study.

qRT-PCR

Total RNA was acquired from serum and tissues using the Trizol (Thermo Fisher Scientific, USA) method. After purity verification, RNA was treated by reverse transcription as a template to get cDNA (Thermo Fisher Scientific, USA), followed by qRT-PCR (Thermo Fisher Scientific, USA). The reaction system: 1 μL cDNA, 5 μL 2×SYBR Green Mix, 0.2 μL ROXII, 0.5 μL upstream, and downstream primers, respectively, and 2.6 μL RNase-free water. The reaction conditions: 94°C/4 min, 60°C/34 s, and 72°C/34 s, 40 cycles in total. Tsingke Biotechnology Co., Ltd. designed and constructed primer sequences. $2^{-\Delta\Delta ct}$ was adopted for calculating the rela-

Table 1. Primer sequence

	F (5'-3')	R (5'-3')
p53	ACCCAGGTCCAGATGAAG	GCAAGAAGCCCAGACG
PKD1	GNSCLCTGAGCTAGCCTANSCLCGCC	ATGCCGTGGATACTTGGA
MAP2K4	TGTGGCACTGGATCAATGAG	GTCTTGCTGACCAATGAGAG
GAPDH	CAATGACCCCTTCATTGACC	TGGAAGATGGTGATGGGATT

Table 2. Clinical baseline data of the research group and the control group

	Research group	Control group	t/x^2	P
Age (years)	54.35±7.40	56.52±8.24	1.573	0.118
BMI (kg/cm^2)	22.27±2.50	21.98±3.31	0.574	0.567
Lesion size (cm)	5.53±1.23	-		
Family medical history				
Yes *vs.* no	15 *vs.* 69	7 *vs.* 43	0.34	0.56
Smoking history				
Yes *vs.* no	22 *vs.* 62	14 *vs.* 36	0.052	0.819
Drinking history				
Yes *vs.* no	17 *vs.* 67	9 *vs.* 41	0.1	0.751
Number of pregnancies				
≤1 *vs.* >1	56 *vs.* 28	37 *vs.* 13	0.794	0.373
Past gynecological history				
Yes *vs.* No	34 *vs.* 50	18 *vs.* 32	0.265	0.607

tive expression (internal reference: GAPDH). Primer sequences are shown in **Table 1**.

Follow-up

Patients were followed up for one year after discharge by outpatient service and telephone at an interval of less than 2 months between each follow-up to understand the patients' survival and disease progress. The deadline was January 1, 2022, and the deadline event was the death of the patient.

Outcome measures

Main outcome measures: Expressions of p53, PKD1, and MAP2K4 in EC and their relationship with the prognosis of patients. Secondary outcome measures: The relationship between p53, PKD1, and MAP2K4 levels in serum and cancer tissues of EC patients, and that between p53, PKD1, and MAP2K4 in serum and pathological features of EC.

Statistical analyses

This study used SPSS 22.0 for statistical processing. Inter-group comparison of counting data in (%) or [n (%)] was conducted using the chi-square test. The inter-group comparison of measured data in ($\bar{x}$ ±s) the independent-samples T test. The Kaplan-Meier method was used for survival rate calculation, and the Log-rank test for survival rate comparison. The associations were analyzed by Pearson correlation coefficient, and the predictive value was analyzed by ROC curve. Logistic regression analysis was used for multifactor analysis. P<0.05 suggested a significant difference.

Results

Comparison of clinical baseline data

To ensure the reliability of the experimental results, we compared the clinical baseline data of the two groups. No notable difference was revealed between the 2 groups in age or BMI (P>0.05) (**Table 2**).

The expression of serum p53, PKD1, and MAP2K4

We quantified serum p53, PKD1, and MAP2K4 in both groups and found that the mRNA expressions of serum p53 and MAP2K4 in the research group were lower than those in the control group. The expression of PKD1 mRNA in the research group was higher than that in the control group (P<0.05, **Figure 1**).

The expression of p53, PKD1, and MAP2K4 in the research group

We quantified p53, PKD1, and MAP2K4 in the tissues of the research group and found that the p53 and MAP2K4 mRNA expressions decreased and PKD1 mRNA expression increased in the cancer tissues of the research group (P<0.05, **Figure 2**).

Associations of p53, PKD1, and MAP2K in serum and tumor tissue

Pearson correlation coefficient analysis revealed positive associations of serum p53, PKD1,

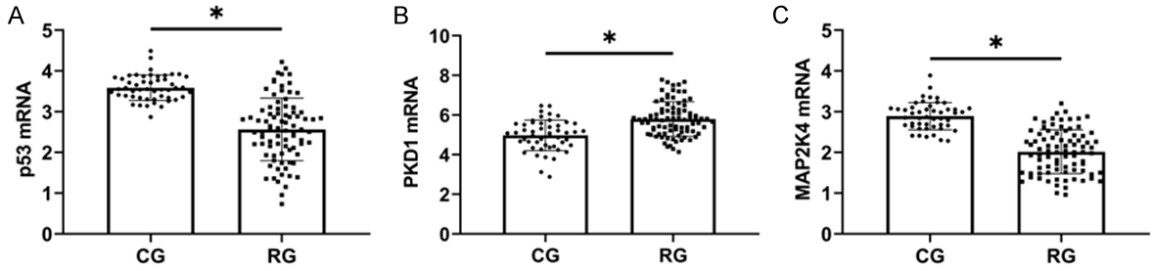

Figure 1. Expression of p53, PKD1, and MAP2K4 in serum (A) Comparison of serum p53 mRNA expression between the research group and control group. (B) Comparison of serum PKD1 mRNA expression between the research group and control group. (C) Comparison of serum MAP2K4 mRNA expression between the research group and control group. *P<0.05.

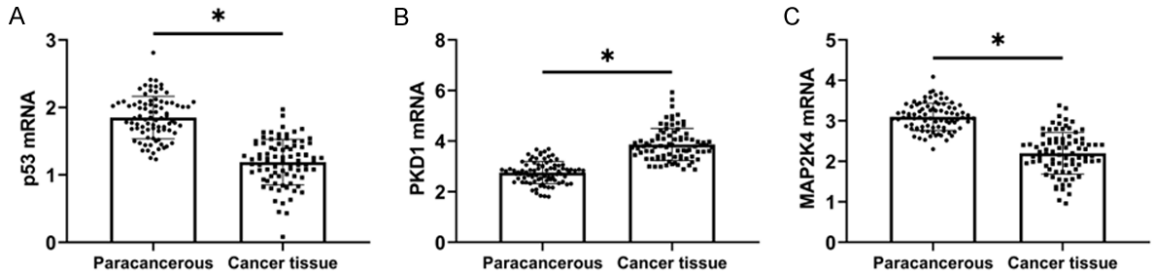

Figure 2. Expression of p53, PKD1, and MAP2K4 (A) Comparison of p53 mRNA expression between cancer tissue and paracancerous tissue in the research group. (B) Comparison of PKD1 mRNA expression between cancer tissue and paracancerous tissue in the research group. (C) Comparison of MAP2K4 mRNA expression between cancer tissue and paracancerous tissue in the research group. *P<0.05.

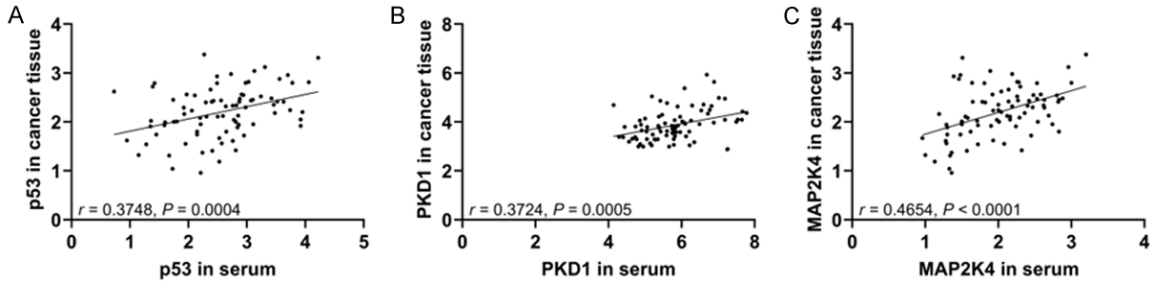

Figure 3. Associations of p53, PKD1, and MAP2K in serum and tumor tissue (A) Association of serum p53 with cancer tissue p53 in the research group. (B) Association of serum PKD1 with cancer tissue PKD1 in the research group. (C) Association of serum MAP2K4 with cancer tissue MAP2K4 in the research group.

and MAP2K with tumor tissue (r=0.375, 0.372, and 0.465, respectively, P<0.05, **Figure 3**).

Relationship between the p53, PKD1, and MAP2K4 levels in serum and EC clinicopathology

No significant difference was revealed in the expression of p53, PKD1, and MAP2K4 among patients of different ages and lesion diameters, suggesting that p53, PKD1, and MAP2K4 were not strongly associated with the age and lesion of EC patients. Patients with lower differentiation, higher FIGO stage, lymph node metastasis (LNM), and deep uterine invasion had lower p53 and MAP2K4 levels and higher PKD1 levels (P<0.05), indicating that all three were relevant to EC differentiation, FIGO stage, LNM, and deep uterine invasion (**Table 3**). Multifactorial analysis manifested that p53, PKD1, and MAP2K were associated with differentiation degree, FIGO stage, lymph node metasta-

Table 3. Associations of p53, PKD1, and MAP2K4 with clinicopathology of EC

		p53	t/P	PKD1	t/P	MAP2K4	t/P
Age (Years)			0.416/0.679		1.329/0.188		-
≤54	41	2.60±0.68		5.67±0.82		2.02±0.58	
>54	43	2.53±0.85		5.92±0.90		2.02±0.50	
Lesion size (cm)			0.354/0.724		1.484/0.142		0.848/0.399
≤5.5	38	2.53±0.80		5.95±0.81		1.96±0.51	
>5.5	46	2.59±0.75		5.67±0.90		2.06±0.56	
Degree of differentiation			5.367/<0.001		3.433/<0.001		5.102/<0.001
Medium-high differentiation	69	2.79±0.67		5.62±0.77		2.17±0.50	
Low differentiation	15	1.79±0.57		6.40±0.92		1.49±0.26	
FIGO stage			5.637/<0.001		5.134/<0.001		8.430/<0.001
I+II phase	60	2.82±0.69		5.53±0.70		2.25±0.44	
III+IV phase	24	1.92±0.58		6.47±0.89		1.44±0.26	
Lymph node metastasis			5.481/<0.001		3.488/<0.001		4.829/<0.001
No	67	2.76±0.68		5.64±0.77		2.14±0.52	
Yes	17	1.77±0.60		6.41±0.97		1.51±0.26	
Deep myometrial invasion			4.312/<0.001		3.986/<0.001		4.713/<0.001
No	70	2.71±0.72		5.64±0.76		2.13±0.51	
Yes	14	1.83±0.56		6.57±0.97		1.47±0.25	

Table 4. Multivariate analysis of pathological characteristics of p53, PKD1, MAP2K4, and EC in serum

		p53	PKD1	MAP2K4
Degree of differentiation	OR	1.622	1.589	1.625
	95% CI	0.642-5.631	0.711-12.135	1.130-2.331
	P	<0.001	<0.001	<0.001
FIGO staging	OR	2.284	1.393	1.801
	95% CI	1.938-4.583	1.036-1.892	1.611-6.812
	P	<0.001	<0.001	<0.001
Lymph node metastasis	OR	1.612	2.626	1.884
	95% CI	1.065-3.606	1.134-5.768	1.342-2.931
	P	<0.001	<0.001	<0.001
Deep myometrial infiltration	OR	1.250	1.107	1.094
	95% CI	0.598-2.527	0.712-2.867	0.714-6.621
	P	<0.001	<0.001	<0.001

sis, and deep muscle infiltration of EC (P< 0.001, **Table 4**).

Predictive value of p53, PKD1, and MAP2K4 expression in serum for prognosis of death in EC patients

By January 1, 2022, we successfully followed 83 patients in the research group, with a success rate of 98.81%. Among them, 11 patients died, resulting in a survival rate within 1 year of 86.75%. The patients who died were assigned to the death group and the survivors to the survival group. A comparison demonstrated that the death group had a notably lower serum p53 and MAP2K4 mRNA levels and a higher PKD1 mRNA level than the survival group (all P<0.05). An analysis based on ROC curves revealed that serum p53 mRNA <2.55 had a sensitivity and specificity of 90.91% and 56.94%, respectively, in forecasting the death of EC patients within one year (P<0.05). Serum PKD1 mRNA >6.24 had a sensitivity and specificity of 63.64% and 80.56%, respectively, in forecasting the death of EC patients within one year (P<0.05). Serum MAP2K4 mRNA >1.75 had a sensitivity and specificity of 90.91% and 72.22%, respectively, in forecasting the death of EC patients within one year (P<0.05) (**Figure 4**).

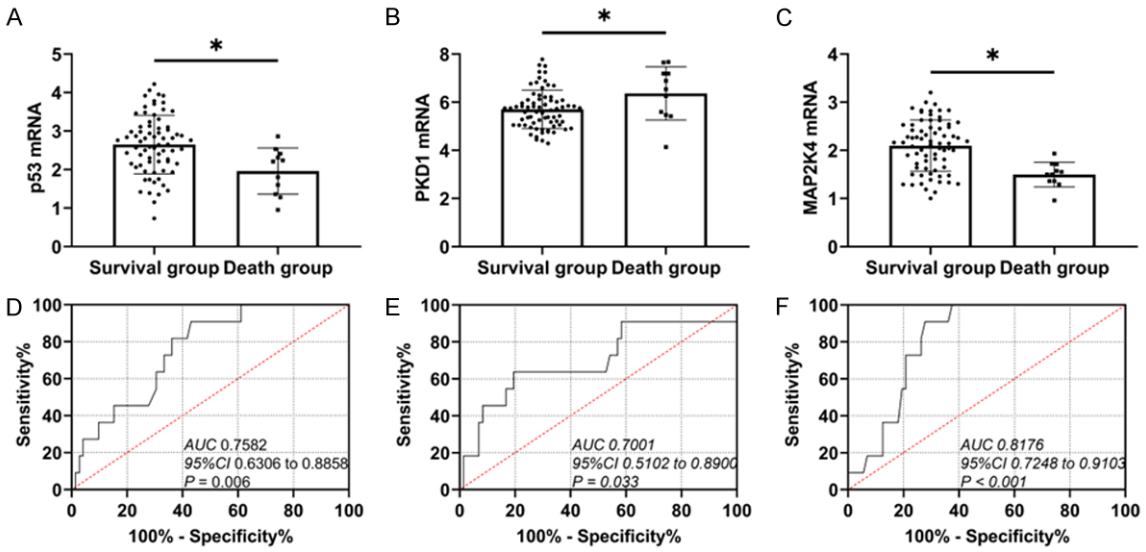

Figure 4. Prognostic value of p53, p53, and PKD1 for the death of EC patients (A) Comparison of serum p53 between the death group and survival group. (B) Comparison of serum PKD1 between the death group and survival group. (C) Comparison of serum MAP2K4 between the death group and survival group. (D) ROC curve of serum p53 in predicting the death of EC patients. (E) ROC curve of serum PKD1 in predicting the death of EC patients. (F) ROC curve of serum MAP2K4 in predicting the death of EC patients. *P<0.05.

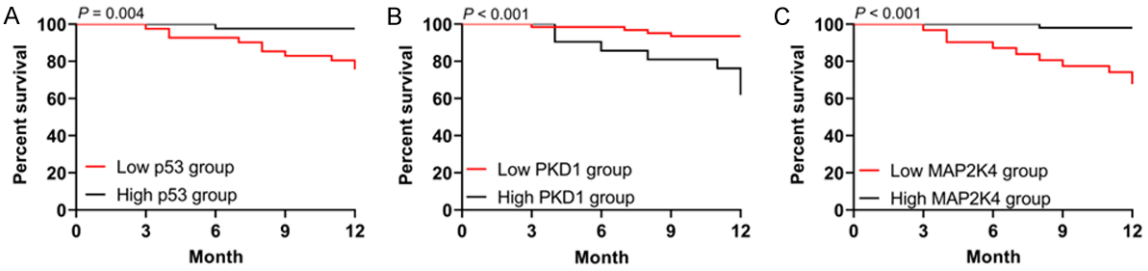

Figure 5. Associations of p53, PKD1, and MAP2K4 with clinicopathology of EC (A) The survival curve of the low p53 expression group and high p53 expression group. (B) The survival curve of the low PKD1 expression group and high PKD1 expression group. (C) The survival curve of the low MAP2K4 expression group and high MAP2K4 expression group.

Association of serum expressions of p53, PKD1, MAP2K4, and prognostic survival of EC

In the light of the cut-off value, patients were assigned to a low p53 expression group (p53 mRNA <2.55) and a high p53 expression group (p53 mRNA ≥2.55), to a low PKD1 expression group (PKD1 mRNA ≤6.24) and a high PKD1 expression group (PKD1 mRNA >6.24), and to a low MAP2K4 expression group (MAP2K4 mRNA <1.75) and a high MAP2K4 expression group (MAP2K4 mRNA ≥1.75). Prognostic survival curves were plotted. The prognostic survival rate in the low p53 group was lower than that in the high p53 group (P<0.05). The low PKD1 expression group showed a higher survival rate than the high PKD1 expression group (P<0.05). The low MAP2K4 expression group showed a lower survival rate than the high MAP2K4 expression group (P<0.05) (**Figure 5**).

Discussion

The research on molecular pathogenicity of tumor diseases can make up for the limitations of early tumor screening in clinical practice, improve the early diagnosis rate of diseases, and lay the foundation for timely therapy of patients [15]. With the lack of reliable evaluation indicators of tumor diseases, the clinical evaluation methods for the disease development of patients are extremely scarce, making

it difficult to control the prognosis of patients [12, 13]. Many of the studies about p53, PKD1, MAP2K4, and tumors are in the investigation of tumor pathogenesis. p53 promotes tumor cell proliferation by regulating immune function [14]. PKD1 deficiency impairs lysosomal activity in a CAPN (calpain)-dependent manner and accelerates tumor development [15]. MAP2K4 interacts with wave proteins to activate the PI3K/AKT pathway and promote BC pathogenesis [16]. Clinical tests regarding all three in EC are instead extremely rare. By analyzing the p53, PKD1, and MAP2K4 levels in EC, this research has more important clinical significance for future diagnosis and treatment.

We detected the expression of p53, PKD1, and MAP2K4 in EC. P53 and MAP2K4 revealed low expression in EC. PKD1 manifested high expression, suggesting that the three are involved in EC occurrence and development. In prior research, the expression of p53, PKD1, and MAP2K4 in cervical cancer or ovarian cancer is consistent with our results [17-19]. As one crucial tumor suppressor gene in the human body, p53 can induce tumor cell apoptosis through multiple signal transduction pathways. Its protein expression product is difficult to detect due to the short half-life and low content. Once p53 has a point mutation, it will lose its anti-tumor effect and convert to oncogenes, resulting in malignant transformation of cells and inducing tumorigenesis [20]. Through immunohistochemical detection, only p53 mutations can be detected, which results in a high expression of p53 in some studies [21, 22]. This study was aimed at the qPCR analysis of wildtype p53. The expression of p53 was low. It is contrary to the result of immunohistochemical detection. PKD1 is mainly found in smooth muscle cells, vascular endothelial cells, or interstitial cells. The increase of its level can promote the activity of gene transcription in cancer nucleus. PKD1 can promote the change of biological characteristics of cancer cells, and then improve the ability of cancer cells to infiltrate and self-proliferate [23]. New studies have pointed out that the increased concentration of PKD1 can improve the breakthrough ability of endometrial glandular cells to the basement membrane, resulting in the enhancement of cancer cell metastasis [24]. MAP2K4 is a factor associated with MAPK signaling pathway. Its regulation of downstream p38 protein or JAPK protein can stabilize the cell cycle of cancer cells and avoid excessive proliferation of cancer cells [25]. Matsumoto et al. have revealed that MAP2K4 can stabilize the activity of tumor stem cells and inhibit the progression of clinical staging of gynecological malignant tumors caused by the activation of tumor stem cells [26]. The detection results of this experiment are consistent with the previous studies on the pathogenesis of p53, PKD1, and MAP2K4, which can once again prove the accuracy of the experiment.

Tumor tissue is the most accurate sample for tumor evaluation. These tissues cannot be collected during early clinical screening because of limited collection conditions. Blood samples have the advantages of convenient collection and long storage time. These blood samples are used in the detection of markers. The expression of p53, PKD1, and MAP2K4 in serum of the research group were positively bound up with that in cancer tissues, which indicated that the expression of p53, PKD1, and MAP2K4 in different samples of EC was cooperative, becoming blood markers of EC. The detection of blood samples has additional clinical significance. The follow-up study was based on the serum expression of p53, PKD1, and MAP2K4 in patients. In the clinicopathological analysis, patients with lower differentiation, higher FIGO stage, LNM, and deep myometrial invasion showed lower p53 and MAP2K4 levels and a higher PKD1 level. This indicated that they were strongly bound up with the differentiation degree, FIGO stage, LNM, and deep myometrial invasion of EC. This confirmed the above experimental results and our point of view, that p53, PKD1, and MAP2K4 participate in the development of EC. Follow-up results indicated p53 and MAP2K4 decreased and PKD1 increased in the dead patients, which demonstrated excellent predictive value for the prognosis of EC. The survival curve was drawn according to p53, PKD1, and MAP2K4, which revealed that the decrease of p53 and MAP2K4 and the increase of PDK1 predicted an increased risk of death. This suggested that the prognosis of patients with EC can be evaluated by the expression of the three factors in the future. Targeted intervention measures can be implemented in time to provide a more reliable guarantee for the prognosis of patients.

Due to the lack of basic experimental analysis, the specific mechanism of p53, PKD1, and

MAP2K4 on EC deserves our further analysis. More case data should be included to analyze the diagnostic value of p53, PKD1, and MAP2K4 in EC. The subjects of this study should be followed up for a longer time to evaluate the impact of the three factors on long-term prognosis.

To sum up, the low p53 and MAP2K4 expressions and the high PKD1 expression in EC cases are all strongly bound up with the development of EC, and can help effectively evaluate the prognosis and survival of patients. They are of crucial research and reference significance for future diagnosis and therapy of EC.

Disclosure of conflict of interest

None.

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