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Second generation sequencing was performed to detect the gene mutation of PIk3CA in gynecological tumors and its relationship with the prognosis of patients

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Abstract: **To investigate the gene mutation of PIk3CA in gynecological tumors and analyze the relationship between PIk3CA and median survival time. Our study included gynecologic tumor patients 110 cases ((40 cases of cervical cancer, 40 cases of endometrial carcinoma, 30 cases of ovarian cancer) from January 2011 1 to August 2019, the First Affiliated Hospital of Sun Yat-sen University and Sun Yat-sen University Affiliated Hospital of Department of Gynaecology. Paraffin tumor samples from patients with postoperative, cut out the white piece, extract DNA, using the second generation sequencing of high-throughput sequencing platform, access to genetic testing results, the Kaplan-Meier survival curve analysis state of PIk3CA gene and the relationship between gynecological malignant tumor patients' overall survival time. In 110 patients with gynecological tumor, 61 cases (55.45%) patients had PIk3CA gene mutations, consensus don't 9745 variants, including 7215 single nucleotide mutation and 2530 missing or insertion mutation, type of PIk3CA mutations in patients with cervical cancer and ovarian cancer gene amplification, highly PIk3CA mutations in endometrial carcinoma was 36.67% (11/30), cervical cancer was 57.5% (23/40), ovarian cancer was 67.5% (27/40), compared with tissue adjacent to carcinoma ($P < 0.05$). The follow-up period was June 1, 2019, and a total of 98 patients were followed up. In cervical cancer and ovarian cancer, the poor prognosis of patients with PIk3CA gene mutation was significantly reduced in the non-mutation group ($P=0.001$), but no correlation was found between PIk3CA gene mutation and the survival time of patients in endometrial cancer. PIk3CA gene is highly extended in gynecological tumors, especially in ovarian cancer and cervical cancer, and is closely related to the poor prognosis of patients, which is great value for guiding clinical prognosis and guiding precise treatment.**

Keywords: PIk3CA; Cervical cancer; Ovarian cancer; Endometrial carcinoma

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1. Introduction

The occurrence of tumor is a process with occult nature, leading to the early detection of disease, and the development of advanced patients with distant metastasis. Therefore, early detection, diagnosis, treatment of malignant tumors, is an important step to improve the prognosis of patients. In recent years, there has been a breakthrough in the targeting of gene and target molecules in gynecologic tumors. Studies have confirmed that phosphatidyl inositol kinase-3 catalytic subunit alpha gene (phosphoinositide 3-kinase catalytic alpha polypeptide gene, PIK3CA) gene mutation caused by PI3K/AKT pathway AKT pathway activation, thus inhibiting the apoptosis of tumor cells, mediated by the occurrence of malignant tumor development[1]. The study confirms that PIK3CA gene mutations can lead to abnormal signals of multiple pathways, and through multiple pathways activate cells. It also promotes the proliferation of cancer cells[2]. Studies have shown that there are PIK3CA gene mutations in gastric cancer and colon cancer[3-4], but the relationship between PIK3CA gene mutations and the occurrence, development and prognosis of gynecological tumors is rarely studied. In this paper, through in-depth analysis of PIK3CA gene mutation in gynecological tumor patients, and to identify its impact on overall survival and prognosis, further to achieve personalized and accurate treatment of gynecological malignant tumor.

2. Materials and methods

2.1 Clinical data

In this study, a retrospective study was conducted to collect clinical data and include 110 cases of gynecological tumor patients admitted to the First Affiliated Hospital of Sun Yat-Sen University and the Sixth Affiliated Hospital of Sun Yat-Sen University from January 2011 to August 2019. 40 cases of cervical cancer were clinical staging: 22 cases in stage I and 18 cases in stage II. Tissue differentiation: 10 cases were highly differentiated, 4 cases were moderately differentiated, 26 cases were poorly differentiated, and 30 cases were endometrial cancer. Age: 41-66 years old, with an average age of

(50.34±6.23) years old. Clinical stage: 18 cases of stage I, 10 cases of stage II, and 2 cases of stage III. Histological grade: 11 cases of high differentiation, 17 cases of medium differentiation, and 2 cases of low differentiation. 40 patients with ovarian cancer, age: 45-74 years, mean (55.44±6.12 years). There were 11 patients with Ic stage, 3 patients with II stage, 21 patients with iiic stage, and 5 patients with IV stage. Histological grade: 32 patients with low differentiation, 6 patients with medium differentiation, and 2 patients with high differentiation. All the patients with gynecologic tumors included in this study were confirmed by surgical resection of the tumor and pathological examination in the hospital. At the same time, the survival time of patients was determined according to the patient's hospitalization case and follow-up information sheet, as well as through telephone follow-up. The last follow-up time set for this study is June 1, 2019.

2.2 High-throughput second-generation full exome sequencing

2.2.1 Tumor tissue samples

Paraffin specimens of gynecological tumor tissues with a thickness of about 5 mm were cut. At room temperature, sections containing tumor specimens were immersed in xylene and anhydrous alcohol for 5 minutes in turn, washed by ionic vesicles, and DNA was extracted from the tissues using a kit.

2.2.2 DNA extraction

All tumor tissue samples were sent to TopGene clinical diagnostic laboratory in Zhongshan city for whole exome sequencing. The genomic DNA was extracted by using the HDQ 96 kit (Omega Bioservices, Norcross, GA, USA). According to the manufacturer's instructions, the specific steps were as follows: (1) cut and mash the tissue in a mortar, add Lysisi Buffer, mix well and continue to add RnaseA. Mix well and let stand for 5 minutes. (2) add Proteinase K, continue to mix, centrifuge in centrifuge for 10min, take the supernatant, add alcohol to mix and centrifuge again at high speed for 1 minute; (3) after mixing with wash buffer, continue to centrifuge and wash the centrifugal column; (4) add tebuffer to collect the liquid in the centrifuge tube and obtain DNA samples.

2.2.3 Nucleic acid quality control

Ultraviolet spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA) was used to detect DNA quality. Qubit fluorometer 3.0 (Thermo Fisher Scientific, Waltham, MA, USA) was used for DNA quantification.

2.2.4 second-generation sequencing

Exons were captured from genomic DNA using the wes-t086v5 panel (iGeneTech, China). PCR products were tested by LabChip GX Touch24 (PerkinElmer). Mgi-2000 was used for contralateral sequencing. The average depth of each sample is ~100X and the reading length is 150bp. Cells with an allele score <5% or 80% of > were screened to reduce discovery of errors due to sequencing errors or a lack of matching.

2.3 Statistical method

Using SPSS20.0 statistical software, chi-square test was used for groups with the total number of samples. Kaplan-meier survival curve was used to analyze the relationship between the mutation status of PIk3CA gene and the median survival time of patients with gynecological tumors. $P < 0.05$ was considered statistically significant.

Table 1. Gene mutation of PIk3CA in gynecologic tumors

GROUP	Case	PIk3CA		χ^2	P
		Mutation type (%)	Wild type (%)		
CC					
Tumor	40	23	17	5.454	0.01
Adjacent	10	1	9		
VC					
Tumor	40	27	13	12.082	0.0005
Adjacent	10	0	10		
EC					
Tumor	30	11	19	5.121	0.01
Adjacent	10	1	9		

3. Results

3.1 Gene mutation of PIk3CA in gynecological tumors

Among 110 patients with gynecological tumor, 61 (55.45%) patients had PIk3CA gene mutation. Consensus was 9745 mutations, including 7,215 single nucleotide mutations and 2530 deletion or insertion mutations. 61 patients with gynecological tumor were

high-frequency mutations of PIk3CA allele, and PIk3CA gene copy number variation was shown in Figure 1. Among them, the PIk3CA mutant gene was highly amplified in cervical and ovarian cancer patients, and the PIk3CA mutation accounted for 36.67% (11/30) in endometrial cancer, 57.5% (23/40) in cervical cancer, and 67.5% (27/40) in ovarian cancer. The differences were statistically significant ($P < 0.05$) compared with the paracancer tissues, as shown in Table 1 and Figure 2.

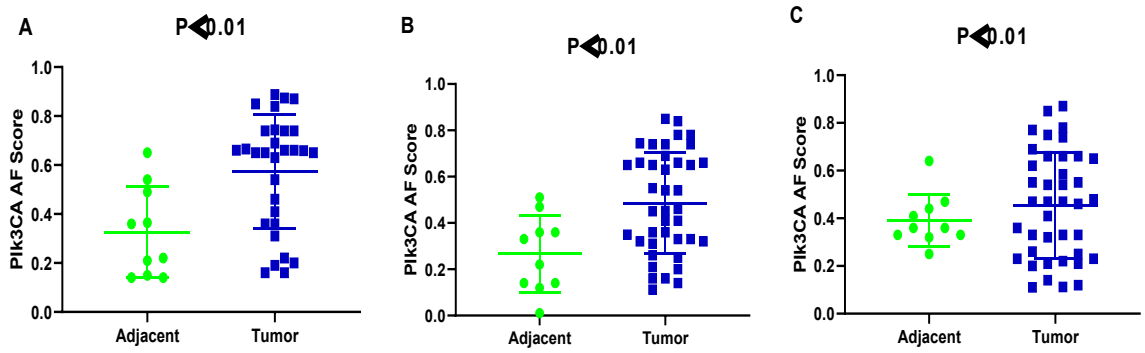

Figure 1. Mutation frequency of PIk3CA allele A: endometrial cancer B: cervical cancer C: ovarian cancer.

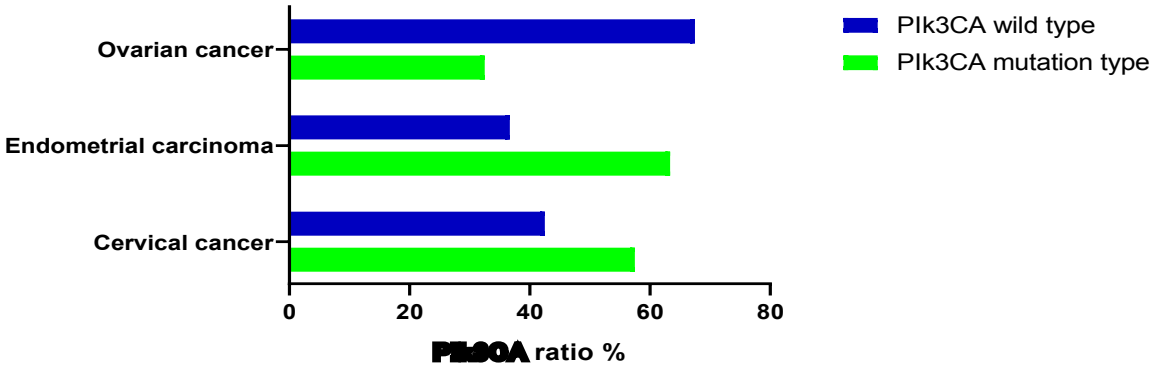

Figure 2. The proportion of PIk3CA gene mutation in patients with gynecological tumors.

Table 2. The relationship between the mutation status of PIk3CA gene and the prognosis of gynecological tumor patients

group	PIk3CA (month)	
	Mutation type (%)	Wild type(%)
VC(n=35)		
Survival time	36.26±0.878	41.15±1.139
95% CI		0.3465-2.225
P		0.0301
EC(n=28)		
Survival time	43.06±0.9858	48.00±1.116
95% CI		0.2698-2.975
P		0.0584
CC(n=37)		
Survival time	36.33±1.125	44.14±1.365
95%CI		0.446-2.834
P		0.0330

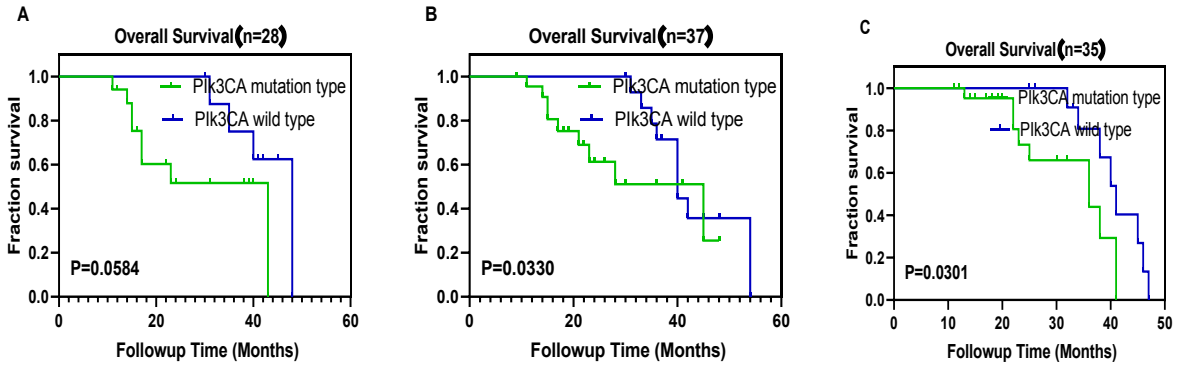

Figure 3. Survival curves of patients with PIk3CA mutation and wild type gynecological tumors, A endometrial carcinoma B cervical carcinoma and C ovarian carcinoma.

3.2 Relationship between the mutation status of PIk3CA gene and the prognosis of gynecological tumor patients

Follow-up deadline is June 1, 2019, this study was summarized as recurrence and death in patients with poor prognosis. Received follow-up of 98 cases, 12 cases were lost to follow-up, cervical cancer, 3 were lost to follow-up, lost to ovarian cancer in 5 cases, 2 cases of ovarian cancer families did not cooperate with telephone follow-up. As a lost to follow-up, 2 cases were lost to follow-up, carcinoma of endometrium PIk3CA mutation type of ovarian cancer the median survival time (42.73 $\pm$ 3.65) months in cervical cancer (57.21 $\pm$ 5.03) months, endometrial carcinoma, the median survival time (46.15 $\pm$ 4.11) months, specific see table 2 and figure 3.

4. Discussion

Malignant tumor is one of the most recognized diseases in the world, which leads to poor prognosis and high mortality. With the improvement of medical technology, the change of working environment and the increase of life pressure, the incidence of malignant tumor is increasing. According to incomplete statistics, the incidence of gynecological tumors in China is still high, and gene mutation is an important factor in the development of tumors[5]. Gene mutations related to cell growth regulation play an important role in the occurrence and development of tumors. As other solid tumors, gynecological tumors, including cervical cancer, endometrial cancer and ovarian cancer, are considered as multi-step abnormal diseases related to tumor suppressor gene inactivation and oncogene activation[6]. At present, scholars believe that they are committed to studying the targeted molecules and biomarkers that can accurately predict gynecological tumors, so as to further improve the existing treatment methods, improve the prognosis of patients and improve the survival rate of patients[7]. PIk3CA is one of the most highly mutated proto-oncogenes in breast cancer. According to the study, PIk3CA gene mutation can lead to overexpression of its target protein PIk3CA and increase the activity of PBK, which leads to abnormal changes in the downstream signaling pathway, which can promote changes in the biological characteristics of cells through multiple mechanisms, and then lead to cancer[8]. PIk3CA gene mutation mainly occurs in two conservative regions, the catalytic domain and the helical domain. The study of such high-frequency hot spot mutation indicates that PIk3CA gene plays an important role in the development of tumors[9]. Abnormal expression of PIK3CA protein can activate the PIK/AKT signaling pathway and promote the development of a variety of solid tumors. In our study, 61 of the 110 patients with gynecological tumors had PIk3CA gene mutation, among which the PIk3CA gene was highly amplified in cervical cancer and ovarian cancer, with statistically significant differences from the paracancer tissues. The results of this study showed that PIk3CA gene mutation was relatively common in gynecological tumors, which also suggested that PIk3CA mutation was one of the important target molecules leading to the development of gynecological tumors. Researchers have suggested that the mutation of PIk3CA gene may be related to the clinical grade of tumor and lymph node metastasis[10], but other studies have pointed out that in colon cancer tissues, the mutation of PIk3CA gene has no correlation with the patient's age, gender, TNM stage, lymphatic metastasis, and general type[11]. Zheng Wenlan[12] study also showed that the different types of malignant tumor, the discovery of PIk3CA gene mutation rate is 11% on average, in cervical squamous cell carcinomas, PIk3CA gene mutation rate is as high as 36%. In cervical squamous cell carcinomas, PIk3CA gene of PI3K protein of normal cervical tissue is low the cervical squamous carcinoma tissues, and with the survival and prognosis of patients

with obvious relationship. However, the current studies on PIk3CA in gynecological tumors are inconsistent in terms of the prognosis of patients in different types of cancers or different studies. PIk3CA gene mutation often occurs in colon cancer, and the mutated sites are mostly exons at position 9 and 20. The relationship between PIk3CA gene mutation and prognosis of breast cancer patients is different in different diseases [13]. In this study, the relationship between PIK3CA gene mutation and patients' prognosis was detected by second-generation sequencing. The results of this study showed that patients with PIK3CA gene mutation in cervical cancer patients had worse prognosis than patients without PIK3CA wild type, and the median survival time was significantly shortened. Scholars believe that the poor prognosis of patients with PIK3CA gene mutation in cervical cancer may be caused by mediating the regulation of malignant proliferation and promoting apoptosis of tumor cells, which may influence the malignant behavior of tumor[14]. This study studied the relationship between PIK3CA gene mutation and patient prognosis at the gene level, which is basically consistent with the research conclusions of other scholars from the protein level. Studies related to literature also showed that in esophageal cancer with PIk3CA mutation, its invasivity was significantly enhanced. The incidence of metastasis was increased, the prognosis of patients was generally poor, and they were prone to drug resistance to a variety of chemotherapy drugs, and even insensitive to radiotherapy, resulting in a generally poor prognosis of patients[15]. Clement[16] believed that PI3K/AKT pathway opening caused by PIK3CA gene mutation was one of the important mechanisms leading to drug resistance to a variety of chemotherapy drugs, so the prognosis of such patients was poor. Other studies have pointed out that the mutation of PIk3CA gene has no obvious correlation with the good or bad prognosis of patients with malignant tumors. But in colon cancer, the mutation of PIk3CA gene usually predicts the poor prognosis of cancer patients[17]. Studies have shown that the mutation of PIk3CA gene opens the PI3K/AKT/mTOR pathway of ovarian cancer, and the prognosis of ovarian cancer patients with PIk3CA gene mutation is generally poor[18]. This study further analyzed the relationship between PIK3CA gene mutation and median survival time of ovarian cancer patients, and the results showed that the average survival time of PIK3CA mutant ovarian cancer patients was significantly shorter than that of PIK3CA wild type. Li Lei[19] also studied patients with ovarian cancer, the survival rate of patients with PIk3CA gene mutation was significantly reduced, but this mutation included amplification and mutation of gene copy number. In our work, the results of this study did not show a significant correlation between the PIK3CA gene mutation in endometrium and the survival of patients, which may be related to the small or limited sample size included in this study. In the future research, the type of research will be further expanded and the follow-up time will be extended, so as to obtain a more reliable basis.

5. Conclusion

PIk3CA gene has a high mutation rate in gynecological tumors, including ovarian cancer, cervical cancer and endometrial cancer, which is an important factor for recurrence of gynecological tumors and poor prognosis of patients. Detection of PIk3CA mutation is helpful to determine the prognosis of gynecological tumors. Although this study analyzed the variation of PIk3CA gene mutation in different types of gynecological tumors and paracancer tissues, and analyzed the relationship between the mutation type and the prognosis of patients, there was no study on the relationship between allele variation number, mutation type and the clinical stage, clinical grade and recurrence of tumors. In addition, it has not been studied whether the PIk3CA gene is mutated after the patients receive targeted therapy.

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