

Downregulation of INPP4B is Associated with Poor Prognosis in Epithelial Ovarian Carcinoma

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Background: INPP4B is a tyrosine-specific phosphatase in the human body, which plays an important role in the developing process of carcinogenesis. However, The correlation between INPP4B and epithelial ovarian cancer is rarely explored. In this study, the expression of INPP4B in human epithelial ovarian carcinoma and normal ovaries was detected, to explore the correlation between INPP4B expression and clinicopathological risk factors of epithelial ovarian carcinoma and to clarify its significance in the developing process of and prognosis of epithelial ovarian carcinoma.

Methods: The expression of INPP4B in various tumors was detected by bioinformatics method, and the expression in epithelial ovarian cancer and normal control group was detected by Elisa. The immunohistochemical method was used in this experiment to analyze the expression of INPP4B in specimens of 100 cases of epithelial ovarian carcinoma and 20 cases of normal ovaries. Analysis of clinicopathological risk factors and related survival analysis was carried out on the expression of INPP4B in 100 cases of epithelial ovarian carcinoma.

Results: The results showed that the positive expressed INPP4B protein in epithelial ovarian carcinoma was significantly less, compared with that in normal ovaries ($P < 0.05$). The expression of INPP4B was significantly associated with many clinicopathologic factors, such as tumor differentiation ($P < 0.001$), FIGO stage ($P < 0.001$), lymph node metastasis ($P < 0.001$) and distant metastasis at recurrence ($P=0.009$), but not with age, pathologic type of tumor, serum CA125 at recurrence and chemotherapy sensitivity.

Conclusion: In epithelial ovarian carcinoma, there is a downregulation of INPP4B expression, which may be related to poor tumor differentiation, late FIGO stage, lymph node metastasis, distant metastasis at recurrence and insensitivity to chemotherapy. Under-expression of INPP4B, lymph node metastasis, FIGO stage, and distant metastasis at recurrence are factors of poor prognostic. The under-expression level of INPP4B may be involved in the progression of epithelial ovarian carcinoma.

Keywords: epithelial ovarian carcinoma, INPP4B, immunohistochemistry: clinical significance, survival

Introduction

Epithelial ovarian carcinoma is one of the gynecological malignant tumors, with the third highest mortality rate after cervical cancer in the world.¹ Epithelial ovarian cancer has a genetic predisposition, and germline BRCA1/2 mutations are the strongest known genetic risk factor for epithelial ovarian cancer. The lifetime risk of ovarian cancer for carriers of germline mutations in the BRCA1 and BRCA2 genes is 35–60% and 12–25%, respectively. BRCA1/2 status can be used to predict a patient's expected survival.²

Although the surgical procedure and chemotherapeutics have been improved, and the overall survival rate of patients with epithelial ovarian carcinoma has improved over the past 50 years, the five-year survival rate is still below 50%.³ The cost of treatment per patient with ovarian cancer remains the highest among all types of cancer. For instance, the average initial cost in the first year can amount to approximately USD 80,000, while the final year cost may escalate to USD 100,000.⁴ In order to mitigate mortality associated with ovarian cancer, public health experts are also seeking an economical and convenient screening strategy. Women with BRCA mutations have a significantly elevated lifetime risk of developing ovarian cancer, typically presenting as high-grade serous carcinoma which is highly aggressive and has a substantial mortality rate. Consequently, current treatment guidelines for epithelial ovarian cancer also encompass

various preventive strategies for patients with hereditary ovarian cancer including genetic counseling for women at high risk and risk-reducing interventions (RRI) such as surgical procedures or chemoprevention through contraceptive medications. However, there still exist several unresolved challenges.

Inositol polyphosphate-4-phosphatase type II (INPP4B) is a phosphoinositol phosphatase, which is a negative regulator of PI3K/AKT signaling pathway.⁵ It is noteworthy to mention that the PI3K pathway is frequently upregulated in epithelial ovarian cancer and plays a pivotal role in chemoresistance and preservation of genomic stability, as it is implicated in numerous processes of DNA replication and cell cycle regulation. Inhibiting the PI3K may result in genomic instability and mitotic catastrophe by reducing the activity of Aurora kinase B, a spindle assembly checkpoint protein, thereby increasing the occurrence of lagging chromosomes during prometaphase.⁶ INPP4B plays different roles and functions in different tumors, and the anti-cancer effect of INPP4B was first reported in breast cancer.⁷ INPP4B is low-expressed in basal cell-like breast cancer, which is related to tumor grade and survival.⁸ In addition, INPP4B also plays the role of tumor suppressor gene in prostate cancer, and the under-expression of INPP4B is closely related to poor prognosis.⁹

Accumulating evidence supports that INPP4B is closely linked to the pathogenesis of cancer, and it is promising to be a new tumor therapeutic target. In this paper, we first applied bioinformatics methods to detect the expression of INPP4B in pan-cancer. Immunohistochemical and Elisa method was used to detect the expression of INPP4B in epithelial ovarian carcinoma and normal ovarian control group, to explore the relationship between INPP4B and the pathogenesis of epithelial ovarian carcinoma. INPP4B has the opportunity to be a new and efficient oncological marker for epithelial ovarian carcinoma. It can be used in early diagnosis and targeted therapy of epithelial ovarian carcinoma, inhibiting the proliferation and metastasis of tumor cells as a tumor suppressor gene, to achieve more efficient treatment. In addition, it can provide evidence for further exploring its mechanism in epithelial ovarian carcinoma, and provide theoretical support and research direction guidance for the pathogenesis of epithelial ovarian carcinoma.

Methods

Gene Expression Analysis

TIMER2 (tumor immune estimation resource, version 2, <http://timer.cistrome.org/>), GEPIA2 (Gene Expression Profiling Interactive Analysis, version 2) tool (<http://gepia2.cancer-pku.cn/#analysis>) and UALCAN (The University of ALabama at Birmingham CANcer data analysis Portal) (<https://ualcan.path.uab.edu/>) was exploited to investigate INPP4B expression profiling spectrum.^{10–12}

Human Tissues

Patients' tissue samples and normal clinical tissue samples were taken from 100 patients with epithelial ovarian carcinoma and 20 patients with normal ovaries. The patients received surgeries at Harbin Medical University Cancer Hospital from January 2011 to January 2013. The paraffin-embedded samples were selected for immunohistochemistry. Inclusion criteria of ovarian carcinoma: 1. Epithelial ovarian carcinoma diagnosed by pathology; 2. No other malignant tumors; 3. The patient had not undergone preoperative neoadjuvant chemotherapy or radiotherapy; 4. The patient's case data was complete. Exclusion criteria: 1) Patients with severe medical complications; 2) Patients with irregular follow-up. A total of 100 patients who met the inclusion criteria were enrolled, aged from 22 to 73 years (median of 53 years). The patients suffered cytoreductive surgery for ovarian carcinoma, followed by six courses of platinum-based chemotherapy, repeated every three weeks. Ovarian specimens from 20 patients in the control group were benign and resected preventively. This study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital (No.: KY2022-68).

FIGO staging of International Federation of Gynecology and Obstetrics was adopted for surgical staging. There were four stages: Stage I, II, III and IV, the former two were defined as early stages, while the latter two were designated as late stages. The classification standards of the World Health Organization were adopted for pathological types of tumors. Tumors were differentiated into well-differentiated (G1) and poorly differentiated (G2/G3). A complete set of data was obtained in patients, including age, pathological type, differentiation degree, FIGO stage, CA125 value at recurrence,

lymph node metastasis, distant metastasis at recurrence, chemotherapy sensitivity, overall survival time (OS) and disease-free survival time (DFS).

All of the patients recruited in the study were followed up regularly until death or five years after the operation. The follow-up interval ranged from 3 to 60 months, 48 months on average. DFS was defined as the interval from the date of surgery to the date of diagnosis of recurrence or distant metastasis, and OS was defined as the interval from the date of surgery to the death of the patient or to the time of data collection.

Elisa

We used an Elisa kit (Abbexa UK) to detect serum from 10 patients with epithelial ovarian cancer and 10 normal controls in Harbin Medical University Cancer Hospital from January to February 2024. Blood samples were placed at room temperature for 2 hours, centrifuged at $1000\times g$ for 20 minutes, and supernatant was collected for later use.

Set standard, test samples and control wells. Aliquot 100 μL of diluted standard into the standard wells. Aliquot 100 μL of Standard Diluent buffer into control (zero) well. Aliquot 100 μL of diluted samples into the sample wells. Incubate for 1 hr at 37 °C. Aliquot 100 μL of Detection Reagent A to each well. Incubate for 1 hr at 37 °C. Wash 3 times. Aliquot 100 μL of Detection Reagent B to each well. Incubate for 30 mins at 37 °C. Wash 5 times. Aliquot 90 μL of TMB Substrate to each well. Incubate for 15 mins at 37 °C. Aliquot 50 μL of Stop Solution. Measure the OD at 450 nm.

Immunohistochemistry

After paraffin embedding, the tissue was sectioned. Then the tissue sections were dewaxed, rinsed with distilled water and soaked in PBS solution for 6 minutes. The sections were rinsed and placed in a pressure cooker containing EDTA repair solution. The sections were boiled for 3 min, and then were taken out and cooled to room temperature to retrieval antigen. The sections were rinsed with distilled water again, and placed in PBS solution for 3 times (4 min each time). The tissue section was incubated with 3% hydrogen peroxide (room temperature, 8 min). Thus, the endogenous peroxidase activity was removed, and the PBS solution was washed 3 times (5 min each time). It was added with a blocking reagent. After incubation (room temperature, 8 min). The ratio of Anti-INPP4B antibody (Abcam USA) to PBS buffer is 1:50, and each slide is slowly dripped with an antibody of 50 μL . Cover all tissues to prevent the formation of bubbles. Add an appropriate amount of diluted primary antibody and incubate at 37 °C for about 2 hours. The section was rinsed with PBS (3 times, 5 min each time). Add biotin labeled secondary antibody working solution (Quick enzyme-linked sheep anti mouse/rabbit IgG polymer 50 μL) and incubate at 37 °C for 20 minutes. It was rinsed with PBS (3 times, 4 min each time). It was added with HRP-SPTM and incubated (37.5 °C, 15 min). It was rinsed with PBS (3 times, 3 min each time). DAB color development: A drop of DAB color reagents A, B and C was added into 1 mL of distilled water respectively. The tissue section was rested at room temperature for 3 to 15 minutes and then observed under a microscope. After satisfaction, the section was fully rinsed with water. The section was re-dyed with hematoxylin, dehydrated, hyalinized and sealed, and then observed under a microscope.

Result Assessment

Two experienced pathologists observed the staining degree of the specimen under a microscope and determined that cells with brown granules in the nucleus or cytoplasm as positive cells. Five visual fields ($\times 400$) were taken from each positive section, and the percentage of 100 positive cells counted in each group was recorded separately. In terms of the proportion of positive cells: $< 5\%$, 5–25%, 26–50%, 51–75% and $\geq 76\%$ were counted as 0–4 points respectively; In terms of the proportion of staining intensity, strong, medium, weak and none of dyeing were recorded as 3–0 points respectively. Finally, the two indexes were scored synthetically, and the scores were divided into four grades: 6–7 for strongly positive, 4–5 for positive, 1–3 for weakly positive, and 0 for negative. The score of each slice was divided into two grades: with 4 points as the boundary. Those with ≥ 4 points were overexpressed, and those with < 4 points were under-expression.

Statistical Analysis

The statistical software used in this paper was spss22.0 (IBM, Armonk, New York, USA). The enumeration data were expressed by composition ratio or rate, and the comparison between groups was carried out by chi-square test. Kaplan-Meier analysis was adopted to draw the survival curves while the Log-rank method to compare the survival rates. Independent prognostic factors including OS and DFS were evaluated by Cox proportional hazard model. Test level $p < 0.05$ suggested statistically significant differences.

Results

Differential Expression of INPP4B Between Tumour and Normal Tissue Samples

Firstly, we compared INPP4B expression levels among tumors and matched normal tissues from 33 cancers using the TIMER database (Figure 1A). Elevated INPP4B expression was observed in BLCA (bladder urothelial carcinoma), CHOL ((Cholangio carcinoma), ESCA (Esophageal carcinoma), KICH (Kidney Chromophobe) et al. INPP4B was low expression in GBM (Glioblastoma multiforme) and KIRP (Kidney renal papillary cell carcinoma). Subsequently, we combined TCGA and GTEx datasets to further validate the INPP4B expression differences in multiple normal tissues and tumor tissues (Figure 1B). INPP4B was significantly low expression in tumor tissues when compared with normal tissues (Figure 2).

The Expression of INPP4B in Epithelial Ovarian Cancer Was Detected by ELISA

The results showed that the expression level of INPP4B in epithelial ovarian cancer was 23.54 ± 3.37 , and that in normal ovarian tissue of the control group was 54.05 ± 4.68 . INPP4B was significantly lower in epithelial ovarian cancer, and the difference was statistically significant ($p < 0.05$) (Figure 3).

Immunohistochemistry Detection of the Expression of INPP4B Protein

INPP4B was expressed in the cytoplasm of both the lesion tissues and the normal ovarian control group. The over-expression rate of INPP4B in epithelial ovarian carcinoma was 30% (30/100). The overexpression rate of INPP4B in normal ovaries was 80.0% (16/20). Immunohistochemical results showed that INPP4B protein was low expressed in epithelial ovarian cancer than in normal ovarian tissue. ($P < 0.05$) (Figure 4).

Relationship Between INPP4B Expression and Clinicopathological Factors

The expression of INPP4B was significantly associated with many clinicopathological factors. There were significant differences in tumor differentiation degree ($P < 0.001$), FIGO stage ($P < 0.001$), lymph node metastasis ($P < 0.001$) and distant metastasis at recurrence ($P = 0.009$), but no significant differences were found among other indexes (Table 1).

Survival Analysis of Patients with Epithelial Ovarian Carcinoma

Comparison of Kaplan-Meier Survival Rate and Comparison of Survival Rate with Log-Rank Method

To explore the potential application value of INPP4B expression, a retrospective analysis was carried out on 100 epithelial ovarian carcinoma specimens. Kaplan-Meier was used to draw survival curves, and the Log-rank method was used to compare survival rates among groups. The results showed that the low expression group of INPP4B had shorter OS and DFS than the overexpression group (Figure 5).

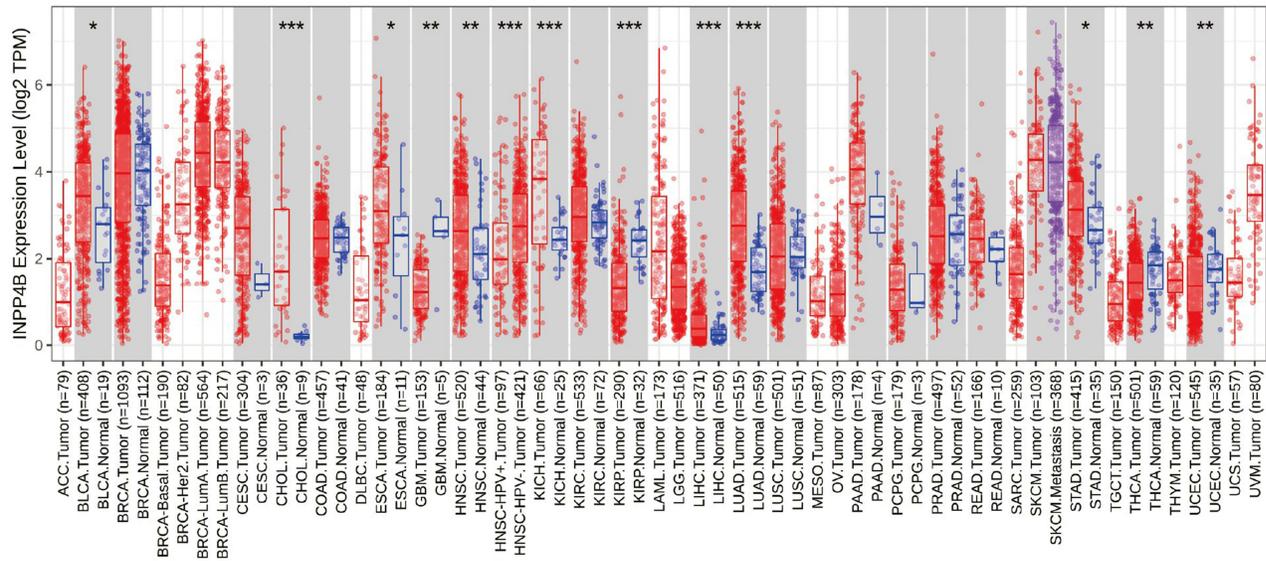
Univariate Analysis of OS

Univariate analysis found that differentiation degree, FIGO stage, lymph node metastasis, distant metastasis at recurrence, chemotherapy sensitivity and INPP4B were the influencing factors of OS, but the interference of confounding factors was not controlled. Thus, multivariate regression analysis was needed to control the interference of confounding factors and find independent influencing factors of OS (Table 2).

Multivariate Regression Analysis of OS

Multivariate regression analysis showed that FIGO stage, lymph node metastasis, distant metastasis at recurrence and INPP4B were independent influencing factors of OS; FIGO stage, lymph node metastasis and distant

A



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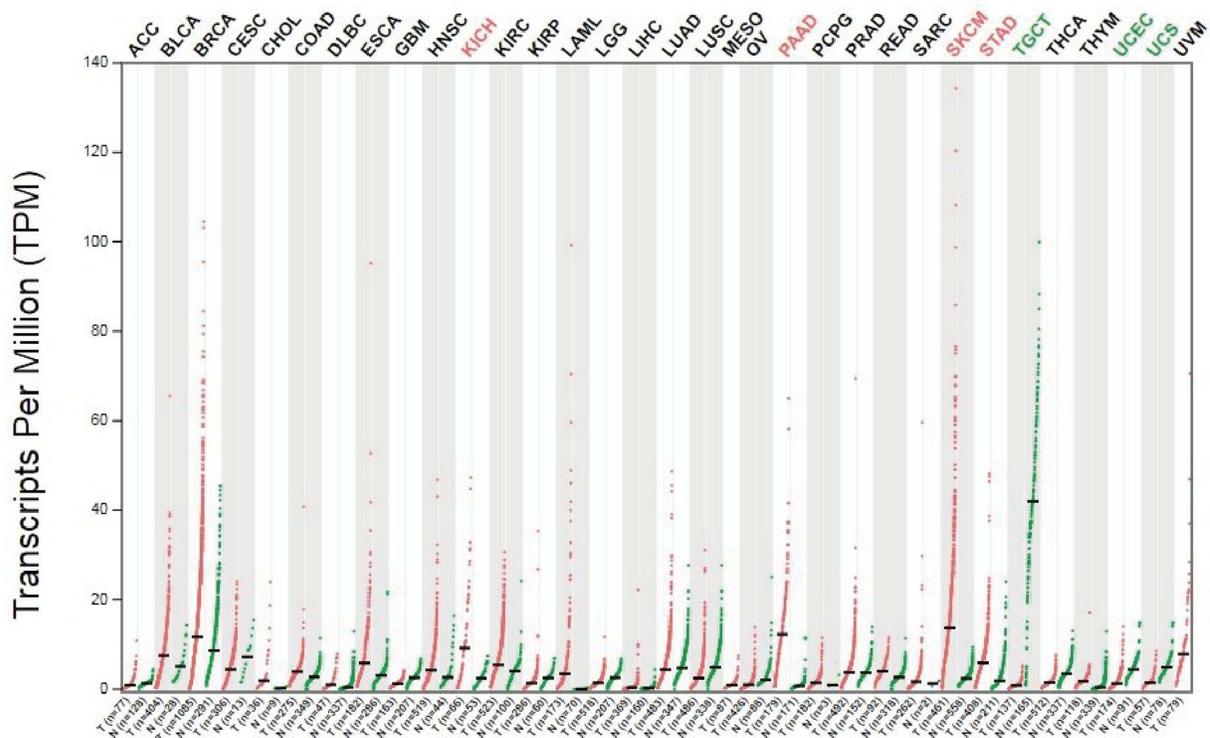


Figure 1 Differential expression of INPP4B in pan-cancer. **(A)** Expression levels of INPP4B in different TCGA tumors from TIMER database. **(B)** Human CLSPN expression levels in different tumor types derived from the GTEx database (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

metastasis at recurrence were independent risk factors, while INPP4B was independent protective factor. This indicates that patients with higher expression survived longer, and other indicators were not independent influencing factors of OS (Table 3).

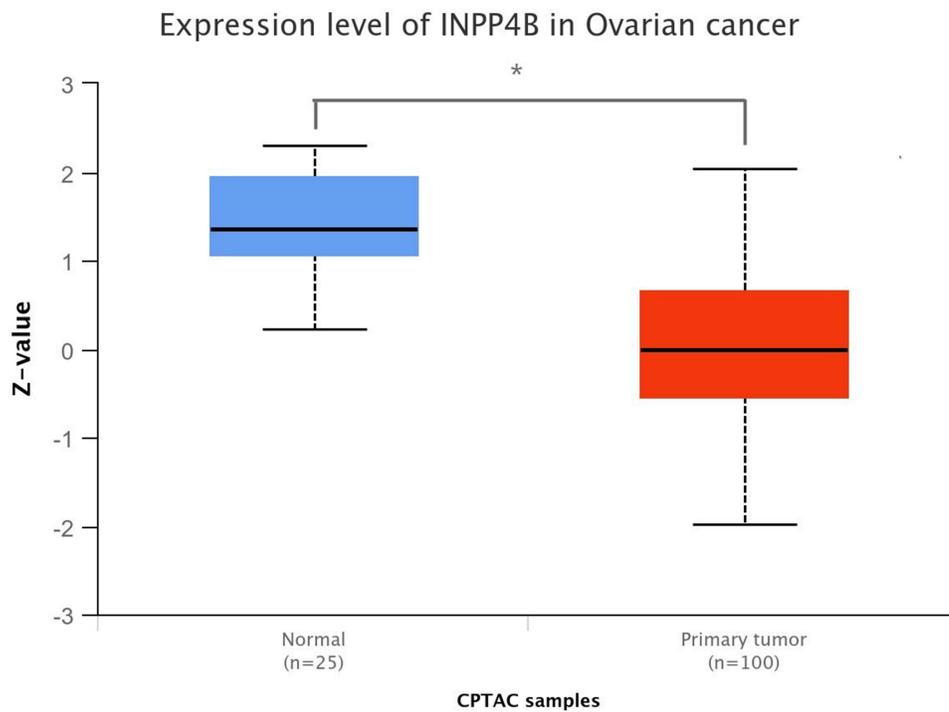


Figure 2 Expression level of INPP4B in Ovarian cancer. (*P<0.05).

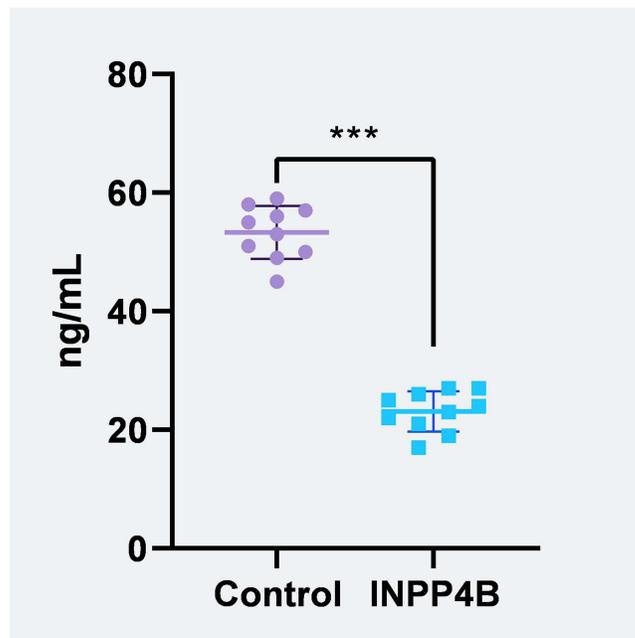


Figure 3 The expression of INPP4B in epithelial ovarian cancer was detected by Elisa. INPP4B was significantly lower in epithelial ovarian cancer. As the result of significance test, ***means p value <0.001.

Univariate Analysis of DFS

Univariate analysis found that differentiation degree, FIGO stage, lymph node metastasis, chemotherapy sensitivity and INPP4B were the influencing factors of DFS, but the interference of confounding factors was not controlled. Thus, multivariate regression analysis was needed to control the interference of confounding factors and find independent influencing factors of DFS (Table 4).

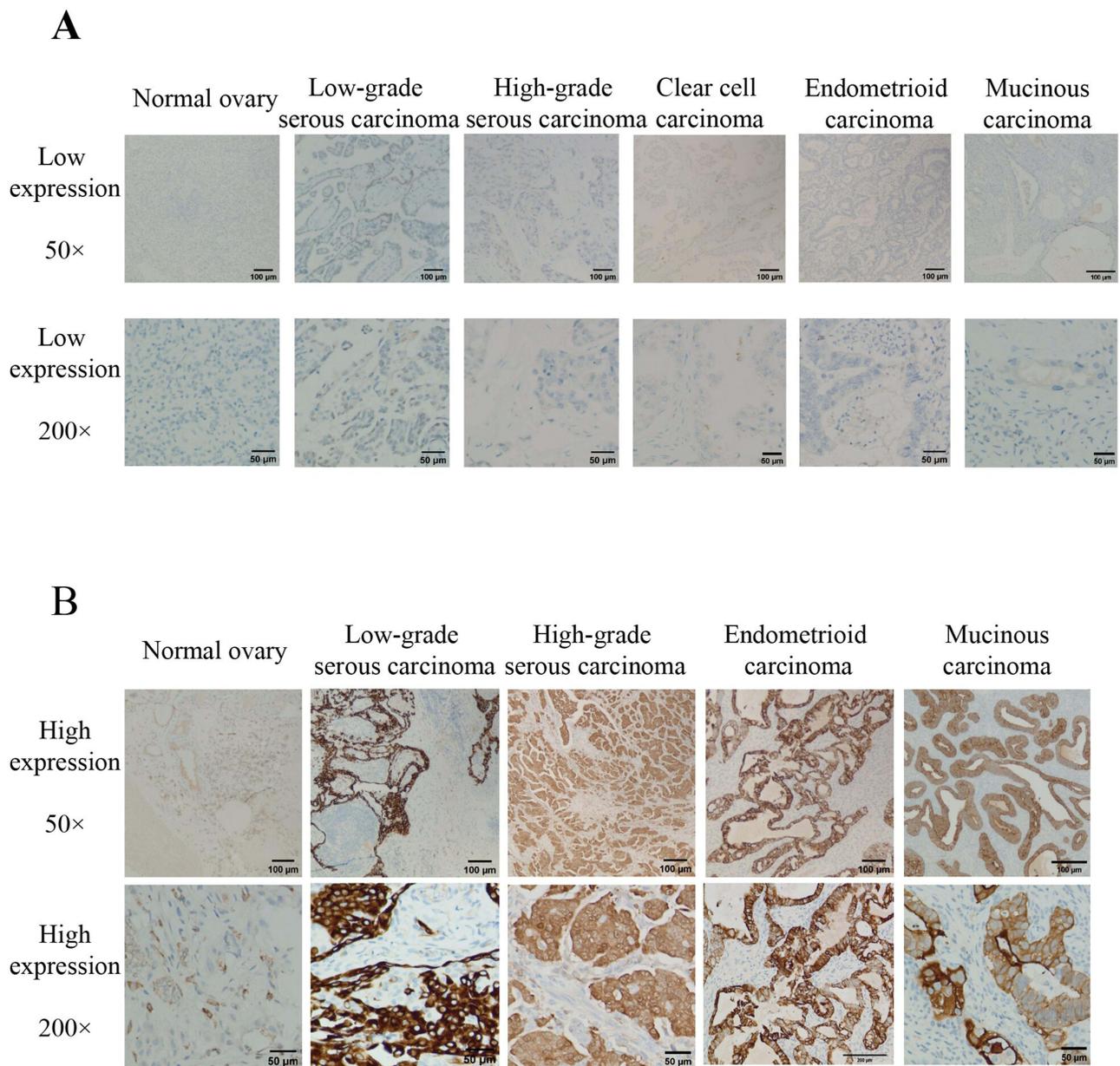


Figure 4 Expression level of INPP4B in epithelial ovarian cancer and normal ovarian tissue detected by immunohistochemistry ($\times 50$ and $\times 200$). **(A)** low expression of INPP4B in epithelial ovarian cancer and normal ovarian tissue detected by immunohistochemistry ($\times 50$ and $\times 200$). **(B)** high expression of INPP4B in epithelial ovarian cancer and normal ovarian tissue detected by immunohistochemistry ($\times 50$ and $\times 200$).

Multivariate Regression Analysis of DFS

Multivariate regression analysis showed that FIGO stage, lymph node metastasis and INPP4B were independent influencing factors of DFS; FIGO stage and lymph node metastasis were independent risk factors, while INPP4B was an independent protective factor. This indicates that patients with higher expression survived longer, and other indicators were not independent influencing factors of DFS (Table 5).

Discussion

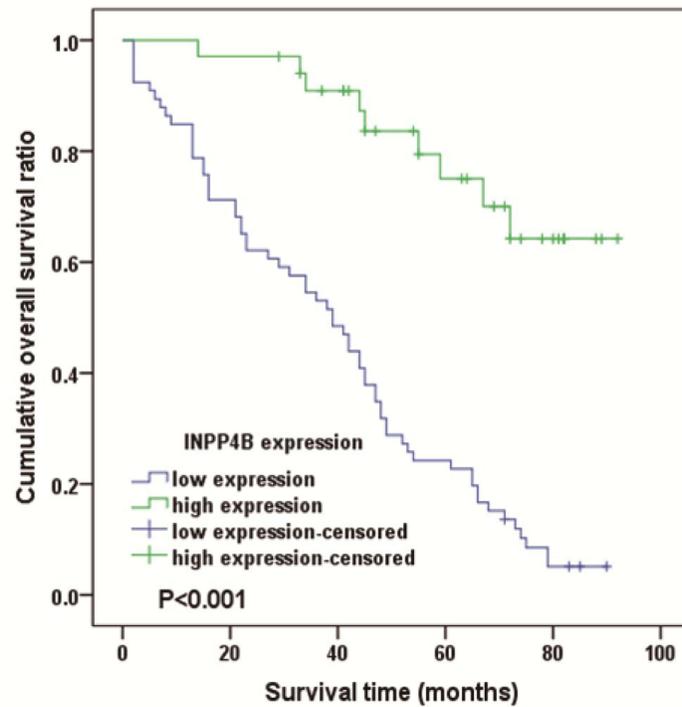
This study aims to explore whether the expression of INPP4B in epithelial ovarian cancer is related to clinicopathological factors and prognosis. We first analyzed the expression of INPP4B in different tumors by bioinformatics methods and found that its expression was increased in BLCA, CHOL and other tumors, but decreased in GBM and KIRP. Further

Table I Relationship Between INPP4B Expression and Clinicopathological Factors

Clinical Parameter	Number of Cases	Low Expression (n=66)	High Expression (n=34)	χ^2	P
Age				3.064	0.080
≤50	30	16(24.2)	14(41.2)		
>50	70	50(75.8)	20(58.8)		
Pathological type				3.887	0.274
Serous	60	40(60.6)	20(58.8)		
Mucosity	19	11(16.7)	8(23.5)		
Endometrioid	17	11(16.7)	6(17.6)		
Clear cell	4	4(6.1)	0(0)		
Degree of differentiation				14.565	<0.001
G1	13	2(3.0)	11(32.4)		
G2/G3	87	64(97.0)	23(67.6)		
FIGO stages				18.747	<0.001
I+II	18	4(6.1)	14(41.2)		
III+IV	82	62(93.9)	20(58.8)		
CA125 value at recurrence				0.105	0.746
≤35	9	5(7.6)	4(11.8)		
>35	91	61(92.4)	30(88.2)		
Lymph node metastasis				13.554	<0.001
No	37	16(24.2)	21(61.8)		
Yes	63	50(75.8)	13(38.2)		
The recurrence was accompanied by distant metastasis				6.819	0.009
No	65	37(56.1)	28(82.4)		
Yes	35	29(43.9)	6(17.6)		
Chemotherapy sensitivity				1.230	0.267
No	21	16(24.2)	5(14.7)		
Yes	79	50(75.8)	29(85.3)		

studies showed that the expression of INPP4B was decreased in epithelial ovarian cancer. This study also detected low expression of INPP4B in epithelial ovarian cancer by Elisa. It continued to expand the sample size and immunohistochemical examination was applied to explore the expression of INPP4B in 100 cases of epithelial ovarian cancer tissues and 20 cases of tissues from the control group. The pathological types of epithelial ovarian carcinoma in this study included high-grade serous carcinoma, clear cell carcinoma, low-grade serous carcinoma, endometrioid carcinoma and mucinous carcinoma. Among them, there were serous carcinoma (n=60), mucinous carcinoma (n=19), endometrioid carcinoma (n=17) and clear cell carcinoma (n=4). Other types of epithelial ovarian carcinoma were not included in this study. There are two different carcinogenic pathways for epithelial ovarian cancer. Type I epithelial cell carcinoma is taken as a relatively indolent and genetically stable tumor, while type II epithelial cell carcinoma is a biologically invasive tumor, which tends to metastasize from the primary site in a small size.¹³ High-grade serous ovarian cancer is the most common epithelial ovarian cancer type, which is about 75% of epithelial ovarian cancer. It develops according to the type II pathway, with p53 and BRCA mutations. Ovarian cancer is one of the gynecological tumors, which has the highest mortality and morbidity.¹⁴ At present, there is a lack of specific and effective methods to diagnose ovarian cancer. Most patients are diagnosed as clinically advanced (stage III or IV), and some even have distant metastasis.¹⁵ Therefore, searching for tumor markers and new drug targets for early diagnosis of diseases is the key to improving the survival and prognosis of patients, with broad application prospects.¹⁶ Proteomics can identify protein targets and pathways related to cancer cell growth and metastasis. Proteomic techniques like protein array analysis and mass spectrometry can promote the anatomy of potential molecular signal events and proteomic characteristics in ovarian cancer. Proteomic analysis of

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B

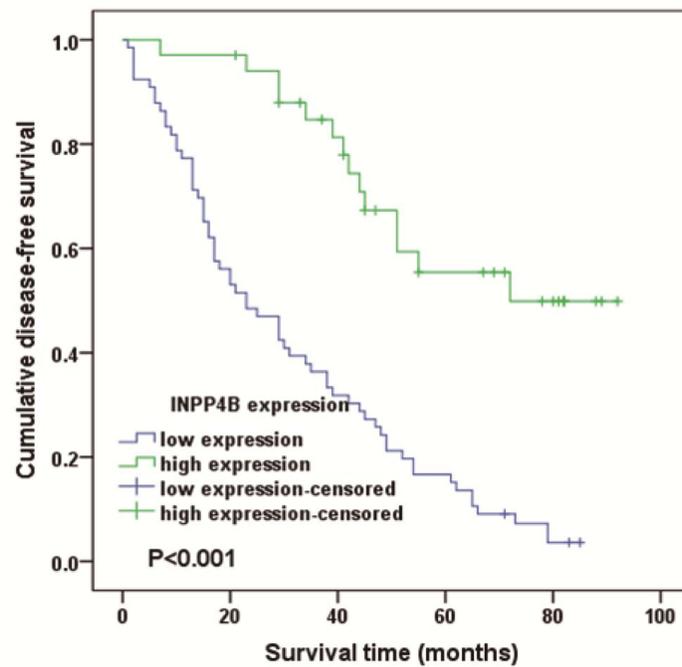


Figure 5 Kaplan-Meier analysis of the relationship between INPP4B expression and survival. (A) disease-free survival (B) INPP4B were highly expressed in patients with epithelial ovarian cancer. Log rank test showed that compared with patients with high expression, patients with low INPP4B staining had significantly lower overall survival rate and recurrence free survival rate.

Table 2 Single Factor Analysis of OS

Clinical Parameter	Number of Cases	OS		Logrank- χ^2	P
		Mean \pm SE	95% CI		
Age				0.419	0.517
≤ 50	30	52.304 \pm 5.952	40.639–63.97		
> 50	70	49.339 \pm 3.367	42.740–55.938		
Pathological type				0.975	0.264
Serous	60	52.924 \pm 3.932	45.216–60.631		
Mucosity	19	43.772 \pm 5.277	33.430–54.114		
Endometrioid	17	51.363 \pm 7.183	37.285–65.441		
Clear cell	4	30.500 \pm 16.993	15.021–63.806		
Degree of differentiation				10.9	0.001
G1	13	77.125 \pm 8.114	61.222–93.028		
G2/G3	87	46.361 \pm 2.970	40.541–52.182		
FIGO stages				22.74	<0.001
I+II	18	86.256 \pm 3.828	78.752–93.759		
III+IV	82	43.202 \pm 2.955	37.411–48.993		
CA125 value at recurrence				0.155	0.694
≤ 35	9	49.444 \pm 8.403	32.975–65.914		
> 35	91	50.596 \pm 3.208	44.307–56.884		
Lymph node metastasis				11.985	0.001
No	37	63.748 \pm 4.286	55.347–72.149		
Yes	63	42.153 \pm 3.548	35.199–49.106		
Distant metastasis occurs when relapse occurs				19.049	<0.001
No	65	59.160 \pm 3.608	52.089–66.231		
Yes	35	34.545 \pm 4.202	26.308–42.781		
Chemotherapy sensitivity				4.046	0.044
No	21	39.758 \pm 5.122	29.720–49.796		
Yes	79	53.244 \pm 3.461	46.460–60.029		
INPP4B				32.654	<0.001
Low expression	66	38.746 \pm 3.123	32.626–44.867		
High expression	34	77.224 \pm 4.213	68.966–85.482		

Table 3 Multivariate Regression Analysis of OS

Clinical Parameter	B	SE	Wald	P	HR	HR95% CI	
						Floor	Upper Limit
Degree of differentiation	0.349	0.706	0.244	0.621	1.418	0.355	5.658
FIGO stages	2.170	0.732	8.797	0.003	8.758	2.088	36.742
Lymph node metastasis	0.707	0.292	5.870	0.015	2.028	1.145	3.592
Distant metastasis occurs when relapse occurs	0.703	0.256	7.548	0.006	2.019	1.223	3.333
Chemotherapy sensitivity	-0.406	0.285	2.025	0.155	0.666	0.381	1.166
INPP4B	-0.980	0.439	4.985	0.026	0.375	0.159	0.887

ovarian cancer and its adaptive response may reveal new treatment options, and this may reduce the occurrence of drug resistance and improve the prognosis of patients. This study analyzed the expression of INPP4B in 100 epithelial ovarian cancer tissues and 20 controls. The expression of INPP4B was low in epithelial ovarian cancer. Further statistical analysis showed that INPP4B expression was significantly correlated with tumor differentiation, FIGO stage, lymph node metastasis at recurrence and distant metastasis. These results also suggested that INPP4B was associated with epithelial

Table 4 Single Factor Analysis of DFS

Clinical Parameter	Number of Cases	DFS		Logrank- χ^2	P
		Mean \pm SE	95% CI		
Age				0.004	0.947
\leq 50	30	42.018 \pm 5.497	31.243–52.793		
>50	70	42.671 \pm 3.530	35.752–49.590		
Pathological type				5.94	0.115
Serous	60	46.586 \pm 3.966	38.813–54.358		
Mucosity	19	32.488 \pm 5.189	22.317–42.659		
endometrioid	17	41.212 \pm 6.630	28.216–54.207		
Clear cell	4	22.75 \pm 11.996	0.000–46.261		
Degree of differentiation				7.704	0.006
G1	13	66.000 \pm 8.477	49.385–82.615		
G2/G3	87	38.675 \pm 2.956	32.882–44.468		
FIGO stages				16.638	<0.001
I+II	18	74.205 \pm 5.847	62.746–85.664		
III+IV	82	35.903 \pm 2.952	30.117–41.689		
CA125 value at recurrence				0.022	0.881
\leq 35	9	45.500 \pm 8.729	28.391–62.609		
>35	91	42.450 \pm 3.213	36.153–48.748		
Lymph node metastasis				11.945	0.001
No	37	56.199 \pm 4.730	46.929–65.469		
Yes	63	34.299 \pm 3.398	27.638–40.960		
Chemotherapy sensitivity				4.023	0.045
No	21	31.862 \pm 4.578	22.889–40.836		
Yes	79	45.483 \pm 3.544	38.538–52.429		
INPP4B				28.165	<0.001
Low expression	66	31.048 \pm 2.918	25.328–36.769		
High expression	34	67.217 \pm 5.013	57.391–77.043		

Table 5 Multivariate Regression Analysis of DFS

Clinical Parameter	B	SE	Wald	P	HR	HR95% CI	
						Floor	Upper Limit
Degree of differentiation	0.052	0.527	0.010	0.921	1.053	0.375	2.959
FIGO stages	1.022	0.447	5.223	0.022	2.780	1.157	6.680
Lymph node metastasis	0.679	0.262	6.713	0.010	1.972	1.180	3.297
Chemotherapy sensitivity	-0.392	0.275	2.032	0.154	0.676	0.394	1.158
INPP4B	-0.982	0.374	6.893	0.009	0.374	0.180	0.780

ovarian cancer, suggesting that INPP4B may act as a tumor suppressor gene in the pathogenesis of epithelial ovarian cancer. The advantage of this study is that the OS and DFS of INPP4B low expression group were shorter than those of INPP4B high expression group through univariate and multivariate analysis. This study shows that INPP4B is an independent prognostic factor of epithelial ovarian cancer, and is related to the progression and metastasis of epithelial ovarian cancer. In previous studies, Gewinner et al¹⁷ found that the low expression of INPP4B in epithelial ovarian cancer was significantly related to the decrease of overall survival and lymph node metastasis, which was consistent with this study. Salmena et al¹⁸ found that abnormal expression of P53 was very common in serous ovarian cancer and was significantly related to the mortality of ovarian cancer. In univariate analysis, low expression of INPP4B in subtypes of endometrial cancer was related to poor survival rate, which was consistent with this study. It is worth noting that they

found that patients with BRCA1 or BRCA2 mutation were more likely to have low expression of INPP4B and abnormal expression of p53. Low expression of INPP4B was more likely to occur in high-grade serous carcinoma and endometrial carcinoma. The main advantage of this research is the large sample size and stratified analysis based on histological subtypes. INPP4B belongs to the phosphatase family, and its main function is to maintain the homeostasis of intracellular phosphoinositol.¹⁹ The role of INPP4B in malignant tumors has been studied.²⁰ INPP4B plays the role of a tumor suppressor gene in some malignant tumors including gastric cancer, breast cancer and colon cancer. Its low expression is associated with poor clinical prognosis.^{21–24} At present, studies on the pathogenesis of epithelial ovarian cancer generally believe that abnormal PI3K/ATK signal is closely related to the pathogenesis of epithelial ovarian cancer and this signal pathway is one of the important signal pathways of cell metabolism and anti-apoptosis. Studies have shown that INPP4B plays a physiological role by negatively regulating PI3K/Akt signaling pathway.^{25,26} In breast cancer, INPP4B inhibits the PI3K/Akt pathway, thus resulting in the effect of a tumor suppressor gene.⁸ INPP4B inhibits PI3K/AKT signaling by converting PI(3,4)P into PI(3)P. ER breast cancer with PIK3CA mutation showed increased expression of INPP4B mRNA and protein, while inhibition of AKT signal transduction. Therefore, INPP4B, as a regulatory factor of PI3K/Akt signal, may act as a suppressor gene in the pathogenesis of ovarian cancer and become a new target for clinical treatment. In conclusion, compared with the normal control group, the expression level of INPP4B in epithelial ovarian cancer tissues decreased significantly. This also indicated that INPP4B was related to epithelial ovarian cancer, and this result also suggested that INPP4B might act as a tumor suppressor gene in the pathogenesis of epithelial ovarian cancer. There are also some limitations and deficiencies in this study. In this study, the number of patients with rare pathological types of epithelial ovarian cancer is small, which may lead to errors in experimental results. In addition, animal and cell experiments have not been carried out in this study for pathogenesis. Therefore, the mechanism of INPP4B in epithelial ovarian cancer remains unclear due to the limitations of the present method.

Conclusion

INPP4B has an under-expression in epithelial ovarian carcinoma, which may be related to poor tumor differentiation, late FIGO stage, lymph node metastasis, distant metastasis at recurrence and insensitivity to chemotherapy. Under-expression of INPP4B, lymph node metastasis and FIGO stage are factors of poor prognosis. The under-expression of INPP4B in patients with epithelial ovarian carcinoma may be related to the progression and poor prognosis of tumor. INPP4B can be used as a new biomarker to analyze the prognosis of epithelial ovarian carcinoma.

Abbreviations

TIMER2, tumor immune estimation resource version 2; OS, overall survival time; DFS, disease-free survival time; IHC, immunohistochemistry; GEPIA2, Gene Expression Profiling Interactive Analysis version 2; UALCAN, The University of ALabama at Birmingham CANcer data analysis Portal.

Data Sharing Statement

The data were all included in this paper. The original data are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The studies involving human participants were reviewed and approved by the Institutional Ethical Committee of Harbin Medical University Cancer Hospital (Ethical Review Number: No.: KY2022-68). The patients/participants provided their written informed consent to participate in this study. The present study fulfils the requirements of the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; JL took part in drafting, WJ revising

or critically reviewing the article; All authors gave final approval of the version to be published; All authors have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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