International Journal of General Medicine

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

EPPK1 as a Prognostic Biomarker in Type I Endometrial Cancer and Its Correlation with Immune Infiltration

Lu Liu[1], Shuang Yuan[1], Shouheng Yao[1], Wenjiao Cao[1,2], Lihua Wang[1]

[1]The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, 200030, People's Republic of China; [2]Shanghai Key Laboratory of Embryo Original Diseases, Shanghai, 200030, People's Republic of China

Correspondence: Lihua Wang; Wenjiao Cao, The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, No. 910, Hengshan Road, Xuhui District, Shanghai, 200030, People's Republic of China, Tel +86-21-18017316018, Fax +86-21-64073896, Email drwanglh0420@163.com; cwj1479@163.com

Purpose: Approximately 20% of patients with type I endometrial cancer (EC) of the uterus experience recurrence and metastasis. However, existing data do not provide sufficient evidence for the utility of protein levels as prognostic biomarkers in type I EC. This study aims to determine whether epiplakin1 (EPPK1) and progesterone receptor (PR) play a role in the recurrence and metastasis of type I EC.

Methods: Following the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) for assessing the quality of biomarker research results, a retrospective analysis was conducted on clinical information and tissue samples of type I EC patients. Protein expression data and clinical data for type I EC were downloaded from The Cancer Proteome Atlas (TCPA) database. We utilized the Kaplan-Meier (K-M) method and Cox proportional hazards regression analyses to evaluate whether epiplakin1 (EPPK1), progesterone receptor (PR) and certain clinical parameters can serve as independent prognostic factors. The Immune Cell Abundance Identifier (ImmuCellAI) and Cancer Immunome Atlas (TCIA) were employed to predict responses to immunotherapy. Immunohistochemistry was carried out to assess the expression of EPPK1 in type I EC.

Results: Type I EC patients with high EPPK1 and low PR expression had higher International Federation of Gynecology and Obstetrics (FIGO) stage, recurrence, and metastasis rates. Furthermore, EPPK1 was identified as an independent prognostic factor, and low expression of EPPK1 was predominantly observed in the POLE ultramutated (POLEmut) group, indicating a favorable prognosis. Additionally, the high EPPK1 expression group had a lower Immune Prognostic Score (IPS), suggesting that the high-expression group may not benefit from immune checkpoint inhibitors.

Conclusion: High expression of EPPK1 is an independent prognostic factor in type I EC patients with low PR expression. It can identify a subgroup of patients at high risk of recurrence. A more aggressive treatment approach is recommended for these patients.

Keywords: type I endometrial cancer, progesterone receptor, epiplakin1, prognostic biomarker, immunotherapy

Introduction

Endometrial cancer (EC) is the common gynecological cancer affecting women worldwide.[1] In recent years, the incidence rate continues to rise, but its mortality rate has risen faster than it,[2] and it ranks second among gynecological tumors in China.[3–5] According to Bokhman's classification, EC is divided into type I and type II.[6,7] Among these, type I EC is known as endometrial endometrioid carcinoma and is the most prevalent type, accounting for approximately 80–90% of cases. Most of these cases exhibit high expression of estrogen and progesterone receptors (ERs and PRs), leading to a more favorable prognosis and a higher 5-year survival rate.[8,9] The majority of type I EC cases are low-risk and are primarily followed up in outpatient settings.[10] However, approximately 20% of type I EC cases are invasive and have a poorer clinical outcome.[11] With the development of the Cancer Genome Atlas (TCGA) molecular subtyping, EC is categorized into four subgroups: POLE ultramutated (POLEmut), p53 mutant (p53abn), mismatch repair-deficient

Received: 16 November 2023
Accepted: 19 April 2024
Published: 29 April 2024

(MMRd), and non-specific molecular profile (NSMP).[12] This classification system has improved the current risk stratification, but the NSMP cases are almost entirely low-grade type I EC.[13] Nonetheless, some of these patients still experience recurrence. Therefore, there is currently a lack of protein-level biomarkers in clinical practice to early predict an adverse prognosis in type I EC.

High expression of PR is positively correlated with the prognosis of EC, including survival rates and survival time.[14] Research has assessed PR expression in 832 samples from EC patients, suggesting that the absence of PR expression may predict lymph node metastasis and shorter disease-specific survival.[15] Low expression of PR in EC is associated with advanced stages, higher grades, and deep myometrial invasion.[16] In the case of type I EC, tumors with lower PR expression tend to be of higher grade,[17] and the absence of PR is a risk factor for recurrence in type I EC patients.[18] Therefore, the loss of PR expression may serve as a biomarker for type I EC patients with a poorer clinical prognosis.

Epiplakin1 (EPPK1) belongs to the plakin family and was initially identified as a 450 kDa human epidermal autoantigen.[19] It is primarily expressed in the liver, small intestine, esophagus, and skin.[20] EPPK1, as a common cytoplasmic junctional protein, is located at the junctions of cell membranes and can integrate the cell's cytoskeleton into muscle fibers. Importantly, it is a part of the EGFR signaling pathway and plays a significant role in the aggregation of the cell cytoskeleton and proliferation signal transduction in tumor cells.[21] While more and more studies are exploring the role of EPPK1 in cancer research and its potential as a biomarker for tumors, comprehensive and systematic research on EPPK1 in endometrial cancer is still lacking.

Currently, the treatment options for endometrial cancer include surgery, radiation therapy, chemotherapy, hormonal therapy, and immunotherapy. Immunotherapy, particularly the use of immune checkpoint inhibitors, has transformed the treatment landscape for many types of cancer, and endometrial cancer is no exception. Immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway have been reported to be effective for patients with advanced or metastatic endometrial cancer.[22]

Therefore, this study aims to determine if PR and EPPK1 expression correlate with recurrence and metastasis of type I EC, as well as to assess the immune cell infiltration associated with EPPK1 and evaluate its responsiveness to immunotherapy.

Materials and Methods

Patient Population

Following the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK),[23] a retrospective follow-up was conducted for 195 type I EC patients who underwent surgical treatment at the International Peace Maternity and Child Health Hospital from September 1, 2015, to January 2, 2019, to assess their 5-year postoperative recurrence status. Clinical and pathological data were collected. The study was approved by the medical research ethics committee of the International Peace Maternal and Child Health Hospital.

Data Sources

Protein expression data for type I EC patients were downloaded from The Cancer Proteome Atlas (TCPA, https://www.tcpaportal.org/tcpa/) database, and clinical information and RNA-seq expression profile data were obtained from TCGA (https://portal.gdc.cancer.gov/) database. By matching sample IDs and utilizing R software, the protein expression data were integrated with clinical information. Patients with incomplete protein expression data or clinical information were excluded, resulting in a final dataset of 300 type I EC patients with complete clinical information and protein expression for further analysis.

Identification and Functional Analysis of Differentially Expressed Proteins (DEPs)

The lower quartile is considered as the boundary point for PR expression.[24] The "limma" package[25] was used to identify DEPs between type I EC patients with high and low PR expression. Further selection was performed based on the $p < 0.001$, and an expression matrix was obtained using the "DESeq2" package. The "pheatmap" package[26] was used for visualizing the heatmap, and the "cluster profiler" package was employed for Gene Ontology (GO), Kyoto Encyclopedia

of Genes and Genomes (KEGG) pathway, and Disease Ontology (DO) enrichment analysis to explore the primary biological functions, signaling pathways, and diseases associated with the DEPs.

Selection and Analysis of Prognostic-Related DEPs

First, using the "survival" package, a univariate Cox regression analysis was performed on the previously selected 59 DEPs. A p value of less than 0.05 was considered statistically significant. Subsequently, the "DESeq2" package was used to choose the differentially expressed DEPs according to |Fold Change| (FC) greater than 1.5 and $p < 0.05$. Combining both pieces of information, the most significant DEP related to prognosis was chosen for further analysis. Patients were divided into high-expression and low-expression groups for EPPK1 based on the median of EPPK1 expression.[27] Then, we used the Kaplan-Meier (K-M) method[28] to evaluate the survival differences between EPPK1 high-expression and low-expression groups in type I EC patients with low PR expression and high PR expression separately, with a significance threshold of $p < 0.05$. This was done to investigate whether EPPK1 can assess the prognosis of type I EC patients with differential PR expression. Subsequently, we conducted K-M method, univariate and multivariate Cox proportional hazards regression analysis, to assess whether EPPK1, along with some clinical parameters, can serve as independent prognostic factors. Stratified analysis was then performed to evaluate the diagnostic ability of EPPK1 in predicting the prognosis of type I EC patients at different levels.

Immune Infiltrating Cells

Tumor-infiltrating immune cells can either promote or exert anti-tumor effects on cancer development. Therefore, quantifying tumor-infiltrating immune cells may reveal the multifaceted roles of the immune system in human cancer. Sangerbox (http://www.sangerbox.com/tool) is a comprehensive resource for systematically analyzing immune infiltration in various cancer types. Using Sangerbox tools, the correlation between 22 immune cell types in type I EC samples was analyzed. The CIBERSORT algorithm was utilized to quantify the proportions of immune cells in mixed cell populations.[29] Analysis involved obtaining RNA-Seq data for type I EC samples from the TCGA database, resulting in an abundance ratio matrix for 22 immune cell types in each sample.[30] The expression differences of each immune cell type between EPPK1 high-expression and low-expression groups were analyzed separately for type I EC patients with low and high PR expression. This was done to further evaluate the relationship between immune cell types and the tumor, as well as their impact on prognosis, with potential implications for immunotherapy.

Prediction of Immunotherapy Response

The Immune Cell Abundance Identifier (ImmuCellAI) is used to predict the response to immune checkpoint blockade based on the abundance of immune cells.[31] It calculates the abundance of immune cells and is utilized in developing response prediction models. The Cancer Immunome Atlas (TCIA) network tool provides comprehensive results of immune gene analysis. Tumor immunogenicity is quantitatively scored from 0 to 10 and referred to as the Immune Phenotype Score (IPS). IPS can be used to predict the response to immune checkpoint inhibitors.[32] Additionally, we conducted correlation analysis between EPPK1 expression and Tumor Mutational Burden (TMB) and the molecular subtypes of type I EC using GraphPad Prism 9.0 software.

Immunohistochemical Staining and Evaluation

In order to investigate the correlation between the expression level of EPPK1 and the prognosis of PR low-expressing type I EC, among the 195 patients we followed up with, after excluding tissue sample losses, 22 randomly selected type I EC tissues with low PR expression were subjected to immunohistochemical (IHC) assessment. Formalin-fixed paraffin-embedded (FFPE) tissue sections were obtained from 22 patients with low PR expression in type I EC. To conduct immunostaining, FFPE tissue blocks were cut into 5-μm thick sections, deparaffinized in xylene, and rehydrated through a graded series of ethanol. Subsequently, the sections were incubated in 0.3% hydrogen peroxide for 25 minutes at room temperature to block endogenous peroxidase activity. Antigen retrieval was performed by microwaving the sections in boiling 10 mM citrate buffer (pH 6.0) for 3 minutes. After blocking with 3% Bovine Serum Albumin (BSA) for 30 minutes at room temperature, the slides were incubated overnight at 4°C with EPPK1 antibody (1:1000 HPA069333,

Merck). Following a phosphate buffer saline (PBS) wash, the sections were incubated with a secondary antibody (1:200, GB23303, Servicebio) for 50 minutes at room temperature. All slides were incubated with Diaminobenzidine (K5007, DAKO) and then washed with distilled water, counterstained with hematoxylin, dehydrated, and coverslipped. A microscope was used to visualize and capture the slides. IHC assessment was performed by two observers who were blinded to the identity of the slides. The scoring method is as follows: The assessment of staining intensity (0–4) is multiplied by the percentage of positive cells for each intensity (0–100%). The final IHC score ranges from 0 to 400. Tumors with a score greater than or equal to 20 (ie, 5%) are interpreted as positive, while tumors with a score less than 20 (5%) are interpreted as negative.[33]

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

RNA was extracted from samples using the RNA-Quick Purification Kit (Yishan, Shanghai, China) and analyzed for RNA content using the NanoDrop ND 2000 (NanoDrop, USA). Subsequently, 500 ng of RNA was reverse transcribed and cDNA amplified using PrimeScript RT Master Mix (Takara, Dalian, China). qRT-PCR was performed using the Hieff®qPCR SYBR Green Master Mix (Yeasen, Shanghai, China) on a QuantStudio 7 Flex system (Life Technologies, USA) following the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control for mRNA expression levels. Primer sequences for qRT-PCR are listed in Table S1. Quantification was performed using the ΔΔCt method.

Cell Culture and Transfection

The EEC cell line (Ishikawa, ISK) was purchased from the American Type Culture Collection (ATCC, VA, USA). ISK cells were cultured in Dulbecco's modified Eagle Medium (DMEM)/F12 (Gibco, Auckland, New Zealand) containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA), 100 μg/mL penicillin, and 100 U/mL streptomycin (Gibco) at 37°C in a 5% CO_2 humidified atmosphere. Transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Two shRNA sequences for the PR and three shRNA sequences for the EPPK1 were used in this study (sh-PR1 and sh-EPPK1-2 showed the highest inhibition efficiency, referred to as sh-PR and sh-EPPK1 in the text, respectively). The sequences used are listed in Table S2.

Cell Proliferation Assay

Cell proliferation was assessed using a Cell Counting Kit-8 (CCK8) following the manufacturer's directions (Yeasen, Shanghai,China). Subsequently, absorbance values were measured at 450 nm using the SpectraMax 190 microplate reader (Bio-Rad Model 680).

Statistical Analysis

Statistical analysis was conducted using R software (version 4.2.2) and SPSS (version 25.0) (SPSS, Inc., Chicago, IL, USA). Clinical and pathological characteristics were analyzed using the chi-square test, and correlation analysis was conducted using Spearman's rank correlation coefficient. And we used the one-way analysis of variance (ANOVA) to calculate the statistical significance of the results. A p value less than 0.05 was considered statistically significant.

Results

Patient Characteristics

A follow-up study was conducted on 195 patients diagnosed with type I EC at the International Peace Maternity and Child Health Hospital from September 3, 2015, to January 2, 2019, to determine the 5-year recurrence rate. It was found that 22 patients experienced recurrence (11.28%). Subsequently, a correlation analysis of clinical and pathological factors was performed, revealing that in type I EC, low PR expression was positively associated with poor prognosis, suggesting that low PR expression serves as a biomarker for poor prognosis in type I EC. FIGO stage (p=0.015), myometrial invasion (p=0.005), lymphovascular space invasion (LVSI) (p=0.048), ER (p<0.0001), PR (p=0.004), and p53 (p<0.0001) were found to be associated with poor prognosis (Table 1).

Table 1 Relationship Between Clinicopathological Features and Recurrence in Patients with Type I EC

Feature	All (n=195)	Non-Recurrence (n=173)	Recurrence (n=22)	p
Age(y)				0.304
≤60	142 (72.82%)	128 (73.99%)	14 (63.64%)	
>60	53 (27.18%)	45 (26.01%)	8 (26.36%)	
Diabetes				0.730
No	173 (88.72%)	153 (88.44%)	20 (90.91%)	
Yes	22 (11.28%)	20 (11.56%)	2 (9.09%)	
Hypertension				0.308
No	132 (67.69%)	115 (66.47%)	17 (77.27%)	
Yes	63 (32.31%)	58 (33.53%)	5 (22.73%)	
Elevated tumor marker				0.335
No	106 (54.36%)	97 (56.07%)	9 (40.91%)	
Yes	77 (39.49%)	66 (38.15%)	12 (54.54%)	
Unknown	11 (6.15%)	10 (5.78%)	1 (4.55%)	
Uterine myoma				0.260
No	119 (61.03%)	108 (62.43%)	11 (50.00%)	
Yes	76 (38.97%)	65 (37.57%)	11 (50.00%)	
FIGO				0.477
I	165 (84.62%)	147 (84.97%)	18 (81.82%)	
II	15 (7.69%)	14 (8.09%)	1 (4.55%)	
III	15 (7.69%)	12 (6.94%)	3 (13.63%)	
IV	0 (0%)	0 (0%)	0 (0%)	
Grade				0.015
I	140 (71.80%)	128 (73.99%)	12 (54.54%)	
II	40 (20.51%)	35 (20.23%)	5 (22.73%)	
III	15 (7.69%)	10 (5.78%)	5 (22.73%)	
Myometrial invasion				0.005
No	51 (26.15%)	46 (26.59%)	5 (22.73%)	
Shallow	117 (60.00%)	108 (62.43%)	9 (40.91%)	
Deep	27 (13.85%)	19 (10.98%)	8 (36.36%)	
Lymphovascular space invasion (LVSI)				0.048
No	141 (72.31%)	129 (74.57%)	12 (54.54%)	
Yes	54 (27.69%)	44 (25.43%)	10 (45.46%)	
Lymph node metastasis				0.744
No	173 (88.72%)	154 (89.02%)	19 (86.36%)	
Yes	11 (5.64%)	9 (5.20%)	2 (9.09%)	
Unknown	11 (5.64%)	10 (5.78%)	1 (4.55%)	
ER				<0.001
No or low	40 (20.51%)	28 (16.18%)	12 (54.54%)	
High	155 (79.49%)	145 (83.82%)	10 (45.46%)	
PR				0.004
No or low	55 (28.20%)	43 (24.86%)	12 (54.54%)	
High	140 (71.80%)	130 (75.14%)	10 (45.46%)	
P53				<0.001
No	93 (47.69%)	87 (50.29%)	6 (27.27%)	
Low	68 (34.87%)	60 (34.68%)	8 (36.36%)	
High	17 (8.72%)	10 (5.78%)	7 (31.82%)	
Wild	17 (8.72%)	16 (9.25%)	1 (4.55%)	

Identification of DEPs in Type I EC Patients with High and Low PR Expression

To distinguish DEPs between type I EC samples with high and low PR expression, we conducted an analysis using the ECC dataset from the TCPA database, which includes 300 samples and 223 expressed proteins. A total of 59 DEPs were

identified ($p < 0.001$, Figure 1a). Subsequently, we filtered the most significant DEPs according to the criteria of $p < 0.05$ and $|FC| > 1.5$. This selection yielded 2 upregulated DEPs and 2 downregulated DEPs (Figure 1b). We also employed univariate Cox regression analysis to identify proteins associated with prognosis (Figure 1c). Through a comprehensive analysis of these results, ER and EPPK1 emerged as differentially expressed proteins correlated with prognosis. Given the strong correlation of ER with PR in type I EC, which has been extensively studied, this study regarded EPPK1 as a significant prognostic differential protein, with the aim of discovering new prognostic biomarkers.

Functional Enrichment Analysis of Differentially Expressed Proteins

To elucidate the primary biological functions and signaling pathways of the DEPs (n=59), we conducted GO, KEGG, and DO enrichment analyses. In the GO analysis, enriched biological processes included gland development, extrinsic apoptotic signaling pathway, regulation of epithelial cell apoptotic process, and cell cycle G1/S transition, among others (Figure 2a). In the KEGG analysis, the PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, and cellular senescence exhibited the highest levels of enrichment (Figure 2b). Additionally, the DO analysis results indicated associations of the DEPs with breast cancer, renal cell carcinoma, lung adenocarcinoma, gastric cancer, ovarian cancer, and endometrial carcinoma (Figure 2c).

Survival Analysis of EPPK1 with Clinical Risk Factors

To further elucidate the prognostic value of EPPK1 in type I EC, we employed a K-M plotter to determine the relationship between EPPK1 and overall survival (OS). Using the median expression level of EPPK1 as the threshold, we divided the samples into high and low expression groups. The results demonstrated that EPPK1 was negatively correlated with OS in type I EC patients (Figure 3a, $p < 0.05$). Using the lower quartile as the cutoff for PR expression, we separated the samples into PR low-expression and PR high-expression groups. Then, based on the median EPPK1

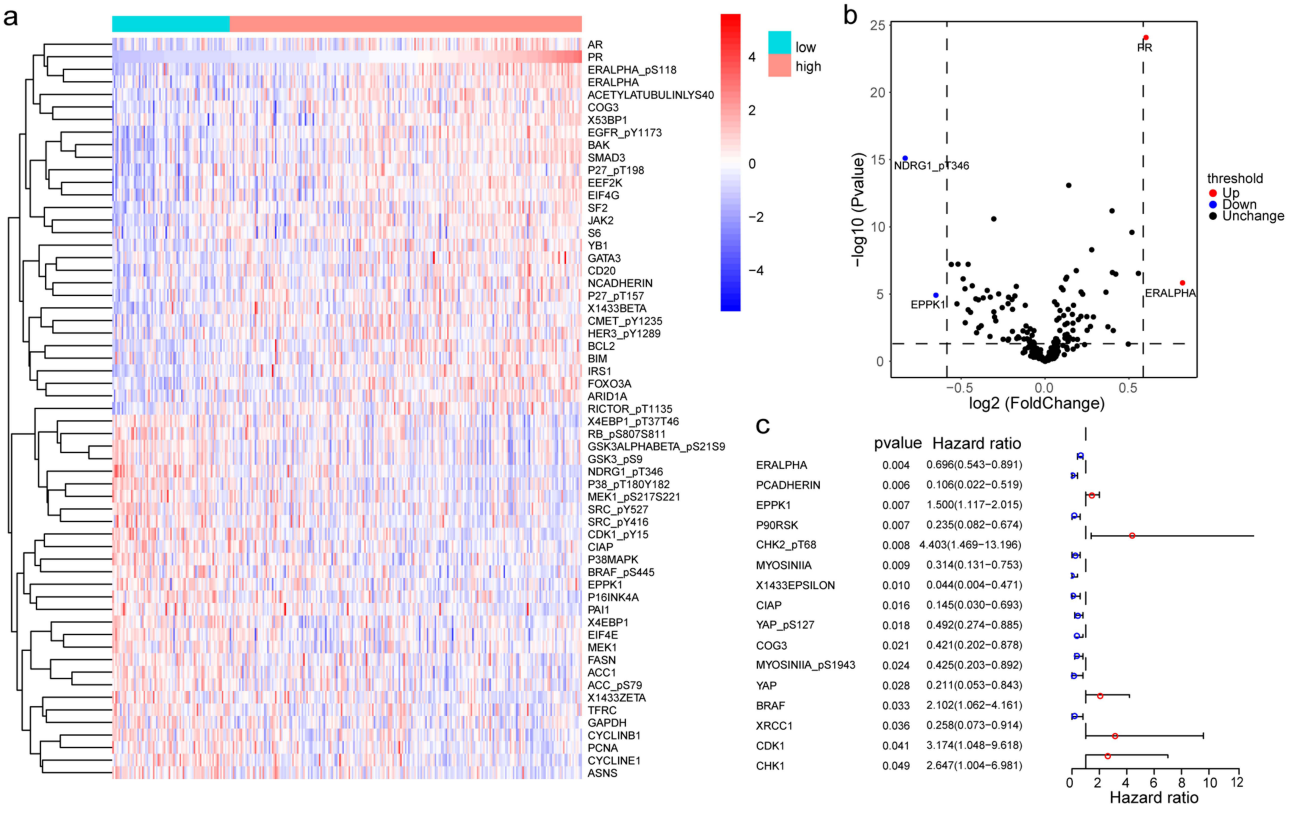

Figure 1 DEPs between type I EC samples with PR high expression and low expression. (**a**) Heatmaps of the DEPs. (**b**) Volcano plots of DEPs. The red dots represent up-regulated DEPs and blue dots represent down-regulated DEPs. (**c**) Forest plot of the Univariate Cox regression analysis in type I EC.

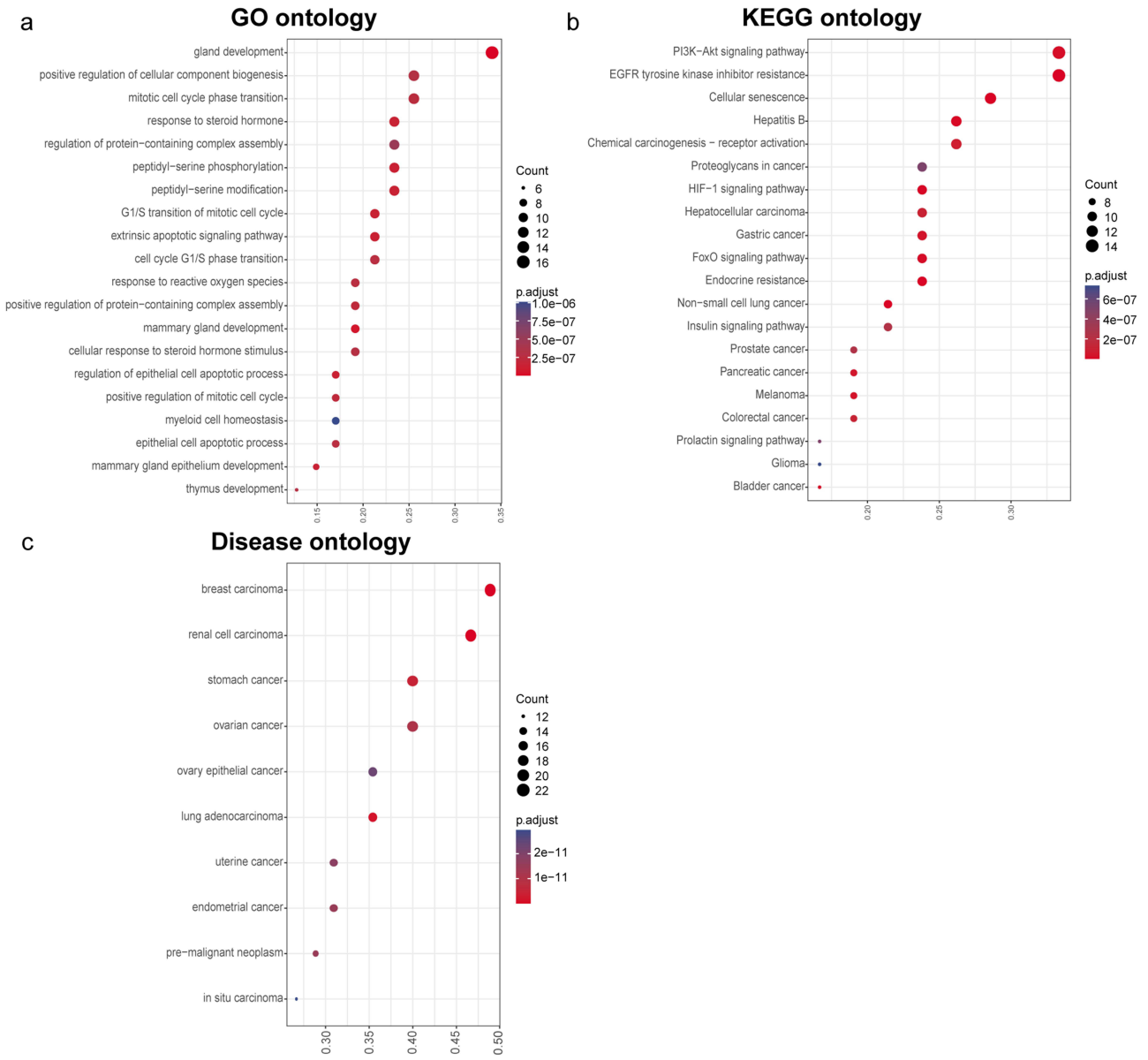

Figure 2 Functional analysis of DEPs. (**a**) GO analysis identified top 20 most significant GO terms. (**b**) KEGG enrichment analysis of DEPs. (**c**) DO analysis identified top 10 most significant DO terms.

expression, we further divided the PR low-expression and PR high-expression groups into EPPK1 high-expression and low-expression subgroups. The results showed that EPPK1 high-expression was associated with poor prognosis in type I EC patients with low PR expression (Figure 3b and c). Additionally, we performed a univariate Cox regression analysis of EPPK1 expression in PR subgroups, which yielded consistent results with the K-M analysis (Table 2).

Subsequently, we analyzed the relationship between conventional clinical risk factors and EPPK1. Using both univariate and multivariate Cox proportional hazards regression analyses, we found that age, FIGO stage, tumor grade, lymph node metastasis, and EPPK1 were independent predictors of prognosis in type I EC patients (Table 3).

Based on age, FIGO stage, tumor grade, and lymph node metastasis, patients were stratified for survival analysis of EPPK1 expression. K-M curves showed that when stratified by tumor grade, there was no significant difference in prognosis related to EPPK1 expression between the groups. However, in comparison to Grade I/II (p=0.15), patients with high EPPK1 expression in the Grade III group (p=0.076) were more strongly associated with poor prognosis (Figure 4a). In the lymph node-negative group, high EPPK1 expression was associated with poor prognosis in Type I EC patients

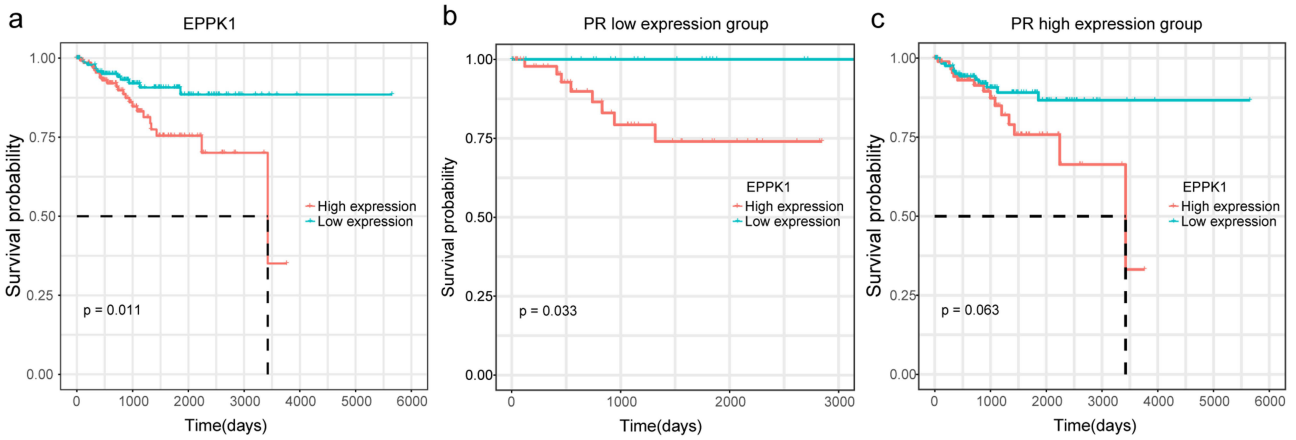

Figure 3 Prognostic gene characteristics of EPPK1 in type I EC patients. (**a**) Relationship between the expression level of EPPK1 and OS was performed using K-M plotter. (**b**) The relationship between EPPK1 expression and OS in the PR low expression group was studied by K-M plotter. (**c**) Association between EPPK1 expression and OS in the PR high expression group was performed with the K-M plotter.

(Figure 4b). We also observed that in the age group >60 years, patients with high EPPK1 expression had worse overall survival compared to low-expression patients. However, the difference in prognosis related to EPPK1 expression was not significant in the age group ≤60 years (Figure 4c). In the analysis of FIGO stage, it was found that in the I-stage subgroup, patients with high EPPK1 expression had significantly lower survival rates than those in the low-expression group (Supplementary Figure 1). Although EPPK1 can be considered an independent prognostic factor for type I EC patients, its value is more significant in specific subgroups.

Relationship Between Prognostic Markers and Tumor-Infiltrating Immune Cells and Immunotherapy

To study the correlation between tumor-infiltrating immune cells and EPPK1 and PR in type I EC samples, we assessed the potential correlations among 22 immune cell subgroups. In Figure 5a, we can observe weak to moderate correlations between the proportions of different tumor-infiltrating immune cell subgroups. Additionally, the CIBERSORT algorithm was used to quantify the proportion of immune cells in each type I EC sample. Figure 5b shows the relationship between EPPK1 expression and immune cells in PR low-expression group. The results indicate that in the PR low-expression group, EPPK1 expression is unrelated to immune cell infiltration. But in the PR high-expression group, the proportion of γδT cells is higher in the EPPK1 low-expression group, while monocytes show higher density in the EPPK1 high-expression group

Table 2 Univariate Cox Regression Analysis of the EPPK1 and Clinical Risk Factors with OS in PR High Expression and Low Expression Groups, Respectively

Parameter	PR Low Expression Group			PR High Expression Group		
	HR	95% CI	p	HR	95% CI	p
Age	1.05	0.99–1.12	0.127	1.05	1.01–1.09	0.008
Grade						
Grade I vs Grade II	1.00	0 - Inf	1.000	5.62	1.26–25.10	0.024
Grade I vs Grade III	3.16×10^{8}	0 - Inf	0.999	5.10	1.15–22.60	0.032
FIGO						
Stage I vs Stage II	1.76	0.18–17.02	0.624	2.49	0.70–8.83	0.159
Stage I vs Stage III	6.29	1.26–31.37	0.025	3.18	1.33–7.57	0.009
Stage I vs Stage IV	2.66	0.28–25.66	0.398	7.87	1.75–35.47	0.007
Metastasis (- vs +)	5.88	1.46–23.71	0.013	2.93	1.23–7.00	0.015
EPPK1	2.81	1.21–6.56	0.017	1.38	0.99–1.92	0.060

Table 3 Univariate and Multivariate Cox Proportional Hazards Regression Analysis of the EPPK1 and Clinical Risk Factors with the OS of Type I EC Patients

Parameter	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	p	HR	95% CI	p
Age	1.05	1.02–1.09	0.002	1.06	1.03–1.10	6.00×10^{-4}
Grade						
Grade I vs Grade II	5.43	1.21–24.26	0.027	4.98	1.11–22.41	0.037
Grade I vs Grade III	5.93	1.39–25.29	0.016	4.69	1.07–20.61	0.041
FIGO						
Stage I vs Stage II	2.26	0.75–6.80	0.149	2.17	0.71–6.62	0.174
Stage I vs Stage III	3.61	1.69–7.72	0.001	3.92	1.25–12.26	0.019
Stage I vs Stage IV	4.28	1.24–14.78	0.022	1.82	0.31–10.49	0.505
Metastasis (- vs +)	3.44	1.68–7.08	0.001	1.54	0.45–5.31	0.492
EPPK1	1.50	1.12–2.01	0.007	1.41	1.04–1.91	0.026

(Supplementary Figure 2a). Furthermore, by selecting optimal cutoff values for patient grouping and using K-M analysis, it was observed that high infiltration of γδT cells was associated with prolonged OS, while high infiltration of monocytes was associated with worse OS (Supplementary Figure 2b). This is consistent with previous results, indicating that low EPPK1 expression is associated with a better prognosis. We evaluated the potential response to immunotherapy for each patient using the ImmuCellAI algorithm. The results showed that patients with PR low-expression and low EPPK1 expression (95%) were more likely to respond to immune checkpoint inhibitors than patients with high EPPK1 expression (93%). However, the difference between the two groups was not particularly significant (Figure 5c).

We further applied TCIA to predict patients' susceptibility to immunotherapy. It was found that the IPS of the EPPK1 low-expression group was higher than that of the high-expression group ($p < 0.05$), suggesting that the low-expression group might be more sensitive to immune checkpoint inhibitors (Figure 6a). However, in type I EC patients with PR low-expression, there was no statistical difference in IPS (Figure 6b). Additionally, as shown in Figure 7a, we found a significant correlation between the expression of EPPK1 and TMB (p=0.008). Similarly, this difference was not significant for type I EC patients with low PR expression (Figure 7b). These results suggest that patients with low EPPK1 expression among type I EC patients may be more sensitive to immunotherapy, but if they also have low PR expression, they may not benefit from immunotherapy.

Subsequently, we conducted an analysis of the expression of EPPK1 and PR in relation to the molecular subtypes of type I EC. In this analysis, PR had the highest expression in the NSMP group, which was significantly different from the other three groups (Figure 7c). Furthermore, samples with low EPPK1 expression were predominantly concentrated in the POLE ultramutated (POLEmut) group. Since the POLEmut represents the best prognosis group, this once again confirmed the strong association of low EPPK1 expression with better prognosis in type I EC (Figure 7d).

Relationship Between EPPK1 and Clinical Pathological Features in Type I EC Patients

To further evaluate the relationship between EPPK1 expression and adverse prognosis in type I EC, among the 195 patients included in our follow-up study, after excluding tissue sample losses, 22 tissue samples from type I EC patients with low PR expression were randomly selected for IHC testing. Subsequently, based on the expression level of EPPK1 in tumor tissue, these 22 patients were divided into EPPK1 low-expression and high-expression groups, with 11 patients displaying low expression and 11 patients exhibiting high expression (Figure 8). We performed a correlation analysis between EPPK1 expression and clinical pathological features (Table 4), including patient recurrence, age, hypertension, uterine fibroids, elevated tumor markers, FIGO stage, tumor grade, myometrial invasion, LVSI, and lymph node metastasis. We found that in type I EC patients with low PR expression, EPPK1 expression was significantly associated with patient FIGO clinical stage (p=0.045) and recurrence (p=0.027).

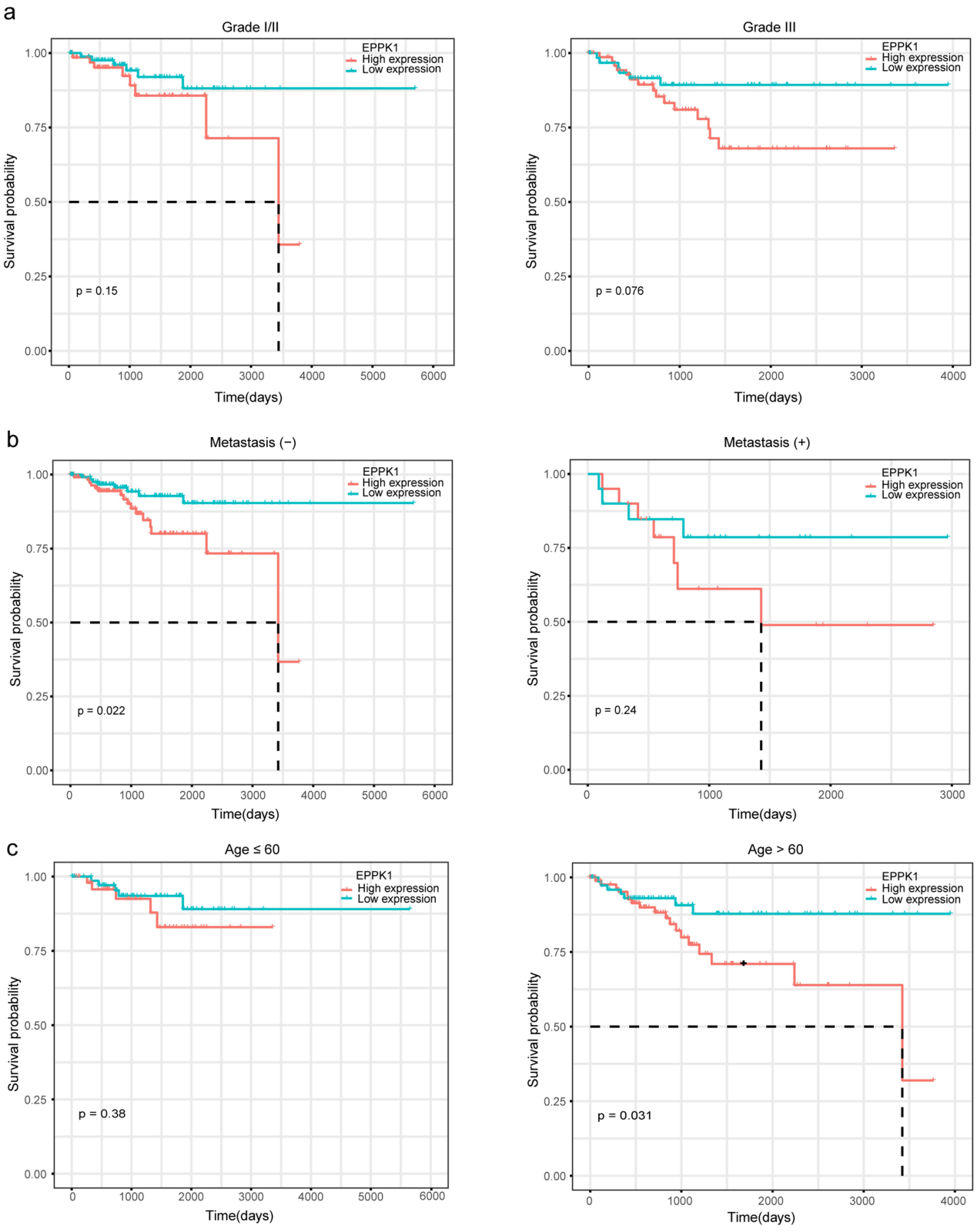

Figure 4 Risk-stratified analysis of the EPPK1 in patients with type I EC. K-M survival analysis of patients in different subgroups. (**a**) Grade I/II and Grade III, (**b**) Metastasis (-) and Metastasis (+), (**c**) Age ≤60 and>60 years.

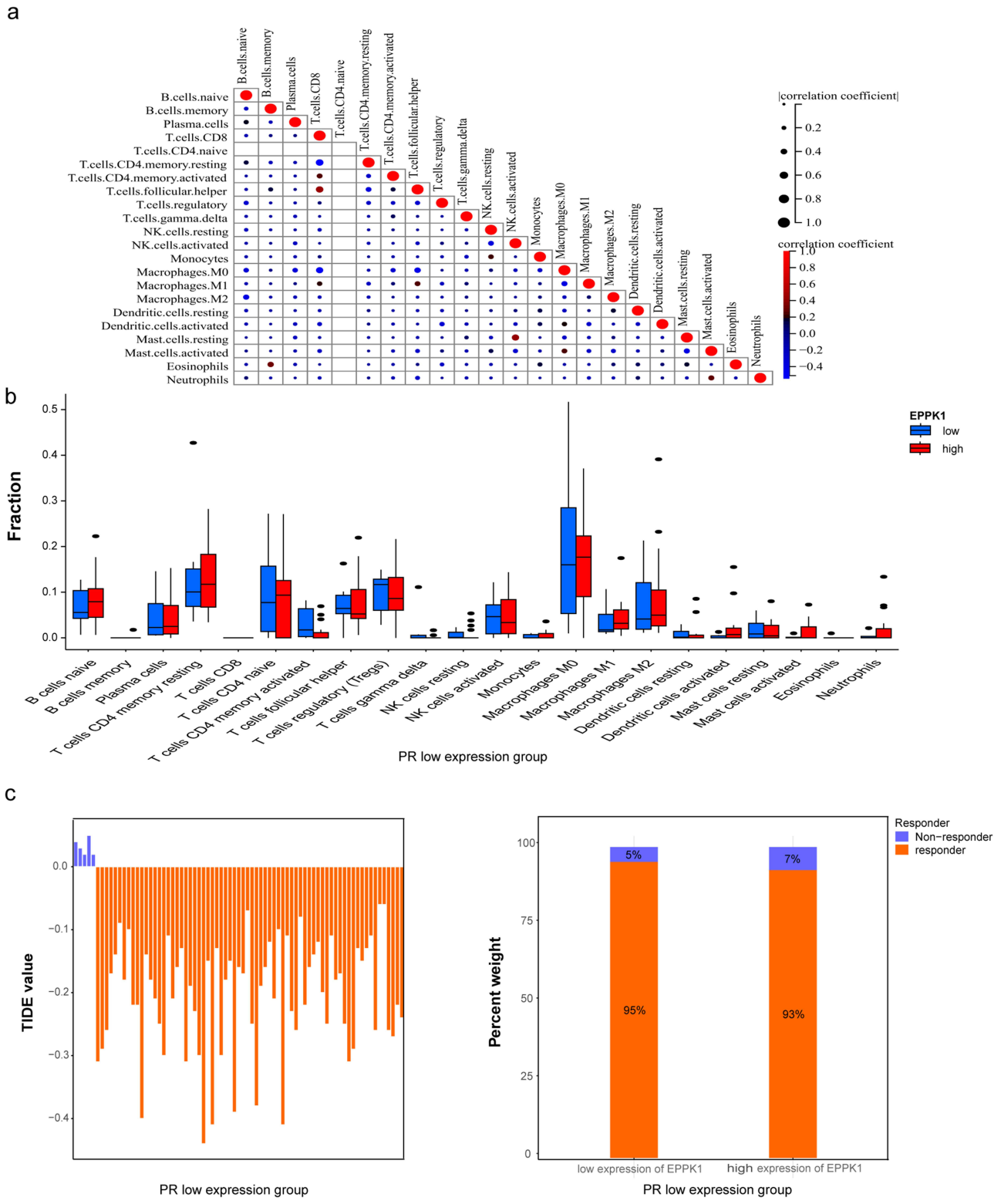

Figure 5 Prognostic features and tumor infiltrating immune cells and immune response. (**a**) The correlation between immune cells. (**b**) Box plot comparing the proportions of immune cells between the high and low expression of EPPK1 in the PR low expression group. (**c**) The sensitive to immunotherapy in the PR low expression group.

Meanwhile, we utilized transfection with sh-PR1 and sh-PR2 plasmids to decrease the expression of PR in the ISK cell line, and employed transfection with sh-EPPK1-1, sh-EPPK1-2, and sh-EPPK1-3 plasmids to lower the expression of EPPK1 in the ISK cell line. We validated their knockdown efficiency using qRT-PCR methods. We selected plasmids

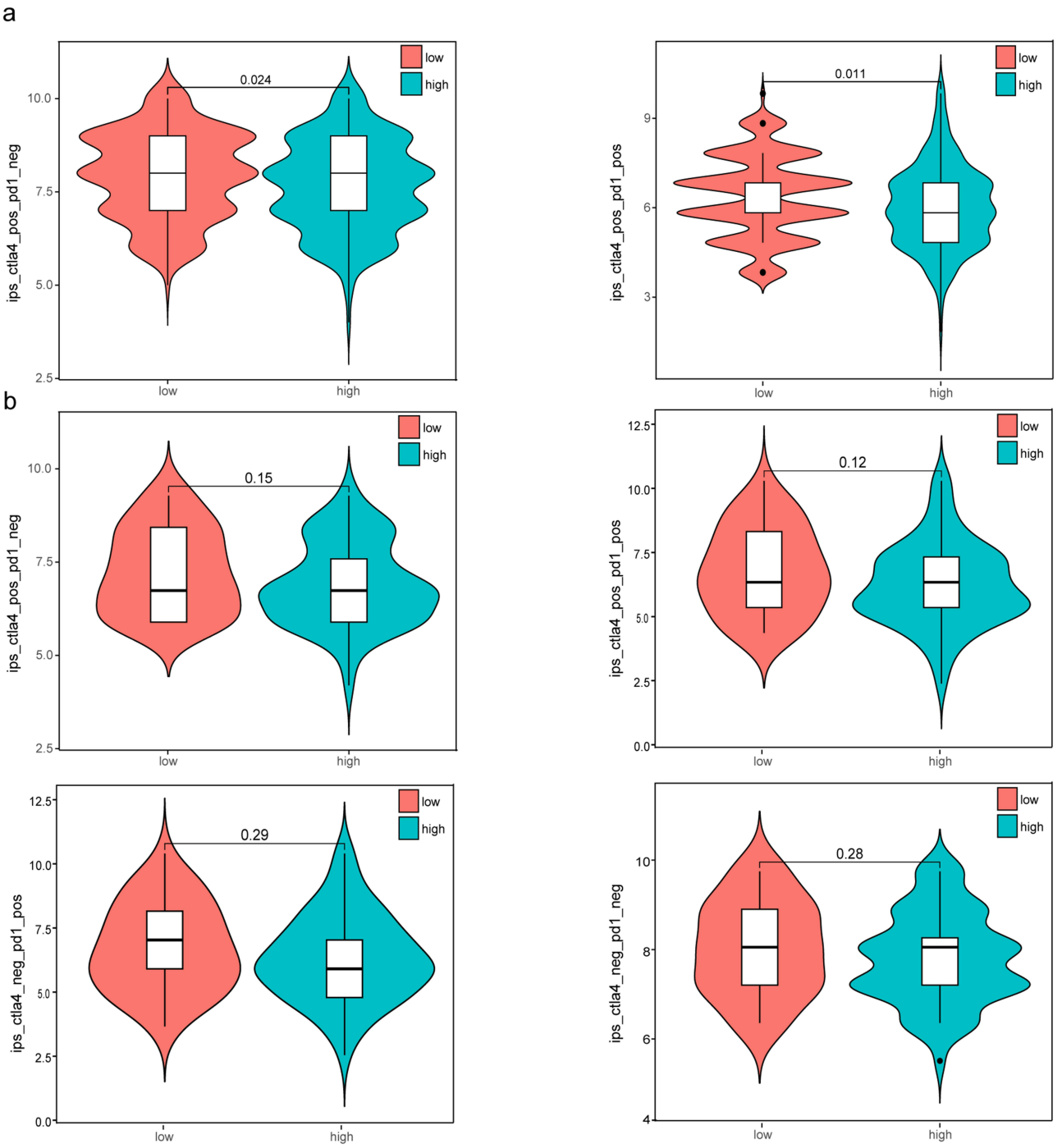

Figure 6 Relationship between EPPK1 expression and immunotherapy. (**a**) The relative probabilities of responding to anti-PD-1/PD-L1 antibody in the low and high expression groups of EPPK1. (**b**) The relative probabilities of responding to anti-PD-1/PD-L1 antibody in the PR low expression group.

with the most significant knockdown efficiency for further experiments, including sh-PR1 and sh-EPPK1-2 plasmids (Figure 9a–d). Results from the CCK-8 cell proliferation assay indicated that knocking down EPPK1 could inhibit cell proliferation compared to ISK cells, and knocking down EPPK1 also inhibited cell proliferation compared to ISK^{shPR} cells (Figure 9e). These experimental results suggest that EPPK1 inhibits the proliferation of EEC cells.

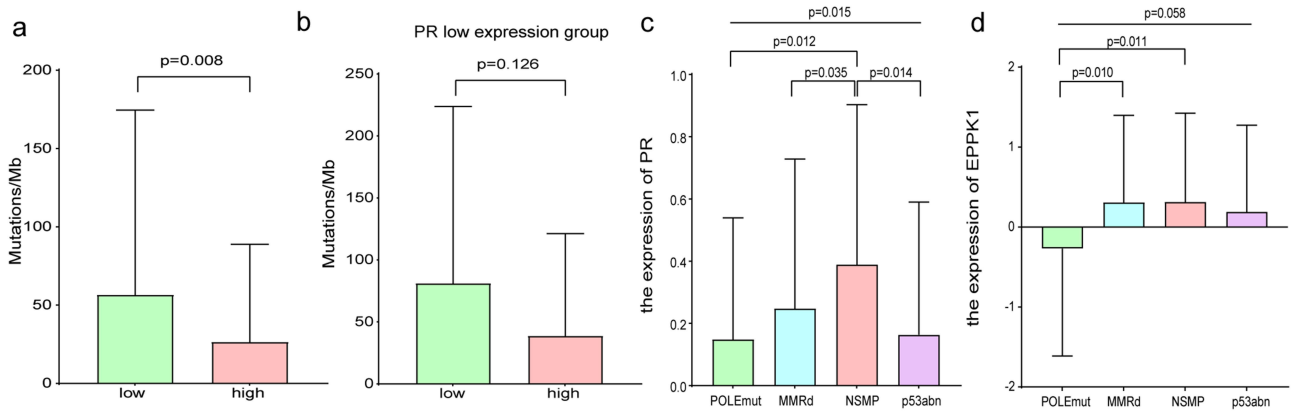

Figure 7 TMB and molecular typing. (**a**) Relationship between TMB and EPPK1 expression. (**b**) Relationship between TMB and EPPK1 expression in the PR low expression group. (**c**) The relationship between PR expression and molecular typing. (**d**) The relationship between EPPK1 expression and molecular typing.

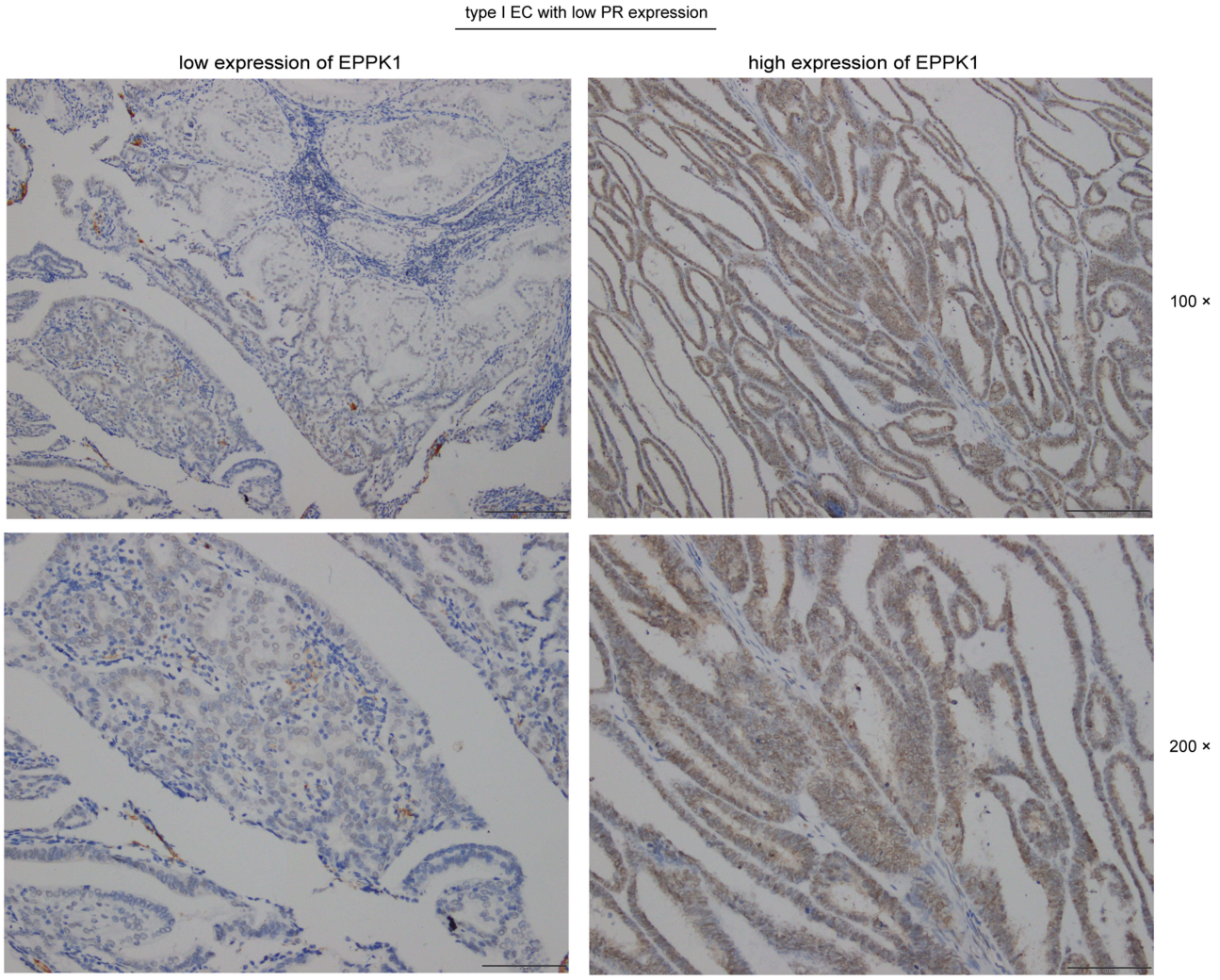

Figure 8 Expression of EPPK1 in type I EC with low PR expression. IHC assays were performed, and the EPPK1 expression levels in type I EC tissues with low PR expression were shown (100x and 200x magnification, respectively).

Discussion

Although type I EC is characterized by a generally favorable prognosis, research has reported significant heterogeneity in disease response.[34] In this study, we assessed the correlation between clinical pathological factors and recurrence in 195

Table 4 The Relationship Between Expression of EPPK1 and Clinicopathological Characteristics of Type I EC (PR Low Expression Group)

Feature	All (n=22)	The Expression of EPPK1		χ^2	p^a	r	p^b
		Low (n=11)	High (n=11)				
Relapse				4.899	0.027	0.471	0.027
No	18 (81.82%)	11 (100%)	7 (63.64%)				
Yes	4 (18.19%)	0 (0%)	4 (36.36%)				
Age(y)				1.222	0.269	0.236	0.291
≤60	18 (81.82%)	10 (90.91%)	8 (72.73%)				
>60	4 (18.19%)	1 (9.09%)	3 (27.27%)				
Hypertension				5.238	0.022	0.488	0.021
No	15 (68.18%)	10 (90.91%)	5 (45.45%)				
Yes	7 (31.82%)	1 (9.09%)	6 (54.55%)				
Elevated tumor marker				4.701	0.030	0.462	0.030
No	13 (59.09%)	9 (81.82%)	4 (36.36%)				
Yes	9 (40.91%)	2 (18.18%)	7 (63.64%)				
Uterine myoma				1.692	0.193	−0.277	0.211
No	9 (40.91%)	3 (27.27%)	6 (54.55%)				
Yes	13 (59.09%)	8 (72.73%)	5 (45.45%)				
FIGO				4.333	0.115	0.431	0.045
IA	16 (72.72%)	10 (90.91%)	6 (54.55%)				
IB	3 (13.64%)	1 (9.09%)	2 (18.18%)				
II	0 (0%)	0 (0%)	0 (0%)				
III	3 (13.64%)	0 (0%)	3(27.27%)				
IV	0 (0%)	0 (0%)	0 (0%)				
Grade				0.386	0.534	0.132	0.557
I+II	19 (86.36%)	10 (90.91%)	9 (81.82%)				
III	3 (13.64%)	1 (9.09%)	2 (18.18%)				
Myometrial invasion				2.329	0.127	0.325	0.139
No or shallow	17 (77.27%)	10 (90.91%)	7 (63.64%)				
Deep	5 (22.73%)	1 (9.09%)	4 (36.36%)				
LVSI				3.143	0.076	0.378	0.083
No	8 (36.36%)	2 (18.18%)	6 (54.55%)				
Yes	14 (63.64%)	9 (81.82%)	5 (45.45%)				
Lymph node metastasis				3.474	0.062	0.397	0.067
No	3 (13.64%)	0 (0)	3 (27.27%)				
Yes	19 (86.36%)	11 (100)	8 (72.73%)				

Notes: p^a is related to χ^2, p^b is related to r.

type I EC patients. Among these factors, tumor grade (p=0.015), myometrial invasion (p=0.005), LVSI (p=0.048), ER (p<0.0001), PR (p=0.004), and p53 (p<0.0001) expression showed significant associations. Previous research has indicated the relevance of PR to type I EC prognosis, and our results are consistent with this. Therefore, based on published proteomic and clinical data of type I EC in the TCPA, we analyzed the DEPs between PR differential expression groups to identify biomarkers associated with adverse prognosis in type I EC due to low PR expression.

In this study, we used univariate Cox regression analysis to validate potential biomarkers associated with type I EC prognosis and identified EPPK1 as a DEP with a negative prognosis, capable of better predicting the prognosis of type I EC patients with low PR expression. As a member of the plakin family, EPPK1 is expressed in various progenitor cells, developing and regenerating cells, especially in pancreatic cancer, where it plays a role in pancreatic development and carcinogenesis through EGF signaling, suggesting its potential as a valuable marker for cancer cell development.[35] EPPK1 is significantly increased in severe pathological changes in cervical tissues, such as cervical intraepithelial neoplasia II–III and cancer, which may be similar to tissue regeneration and development in injured tissues or the

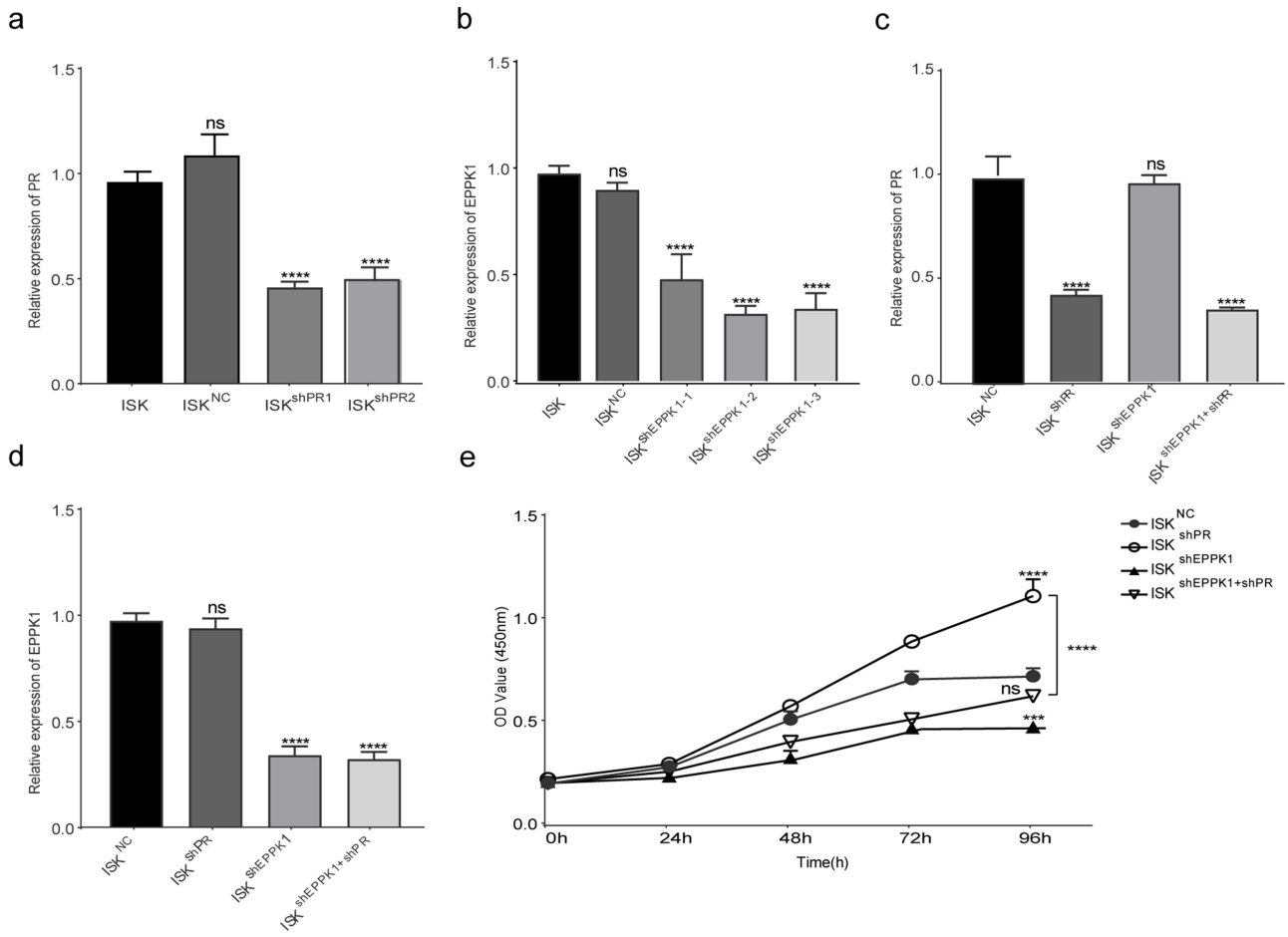

Figure 9 Relative expression level of PR or EPPK1 in ISK cell lines. (**a**–**d**) qRT-PCR showing the expression of PR and EPPK1 in ISK cell lines as well as in ISK cell lines with knockdown of PR, EPPK1, or both. (**e**) CCK-8 assay was performed to determine the ability of proliferation in ISK cells transfected with sh-PR or/and sh-EPPK1. ***p < 0.001; ****p < 0.0001.
Abbreviation: ns, nonsignificant.

upregulation of EPPK1 promoting the occurrence and development of tumors. Ma et al discovered a significant correlation between the increased co-expression of Eppk1 and KLF5 using immunofluorescence staining and the occurrence of tumors in cervical tissues. Additionally, EPPK1 upregulation can promote the proliferation of cervical cancer cells by activating the p38 signaling pathway.[36] Furthermore, EPPK1 can activate the PI3K/AKT signaling pathway and significantly enhance the progression of esophageal squamous cell carcinoma cells, including cell growth, migration, invasion, and epithelial-to-mesenchymal transition.[37] Yang et al showed that EPPK1 knockdown mediated by lentivirus and the addition of dihydrotestosterone significantly inhibited the proliferation and invasion of bladder cancer cells in vitro. They revealed that dihydrotestosterone interacted with the EPPK1 protein, which activated downstream transcription factor c-JUN through the p38 MAPK signaling pathway, promoting JUP expression and increasing cell proliferation and invasion.[38]

Although emerging evidence suggests that EPPK1 is involved in the progression of various cancers, such as liver cancer,[27] cervical cancer,[39] bladder urothelial carcinoma,[40] and esophageal cancer,[37] and is associated with poor prognosis in cancer, its role in type I EC remains unknown. Our results indicate that age, FIGO stage, tumor grade, lymph node metastasis, and EPPK1 can independently predict the prognosis of type I EC. Furthermore, EPPK1 has a high predictive value for the OS of type I EC patients with low PR expression. Clinical sample analysis revealed correlations between EPPK1 and recurrent prevention, hypertension, elevated tumor markers, and FIGO stage in type I EC patients with low PR expression. Additionally, there has been considerable research on the impact of immune infiltrating cells, the immune microenvironment, and immunotherapy on tumor development and treatment,[41] our study results suggest a strong association between low

EPPK1 expression and better responses to immunotherapy. Furthermore, we conducted an analysis of the correlation between the expression of EPPK1 and PR and the molecular subtypes of type I EC, finding that samples with low EPPK1 expression were predominantly concentrated in the POLEmut group. Based on these results, we believe that the upregulation of EPPK1 and the loss of PR expression are biomarkers of poor prognosis in type I EC, and patients with high EPPK1 expression may not benefit from immunotherapy. However, further research is needed to explore the signaling pathways through which EPPK1 upregulation leads to poor prognosis in type I EC with PR loss expression.

Our study has several limitations. Firstly, the data is based on the TCPA database and a limited clinical patient cohort and has not been validated in other databases. Although we have used clinical samples from the International Peace Maternity and Child Health Hospital for validation, the sample size is small. Secondly, our study is based solely on retrospective analysis. Finally, further molecular biology experiments are needed to explore the potential functions and mechanisms of EPPK1.

Conclusion

The risk estimation for type I EC is based on preoperative and postoperative factors. EPPK1 could be a valuable additional tool to help identify subgroups of type I EC patients with a high risk of disease progression. Additionally, high EPPK1 expression may indicate poor responsiveness to immunotherapy, suggesting the need for more aggressive and effective treatment options. However, prospective studies may be required to clarify the value of EPPK1 in predicting recurrence and to elucidate its potential mechanisms in the onset of type I EC.

Abbreviations

EC, Endometrial cancer; REMARK, Reporting Recommendations for Tumor Marker Prognostic Studies; TCPA, The Cancer Proteome Atlas; EPPK1, Epiplakin1; PR, Progesterone receptor; ImmuCellAI, Immune Cell Abundance Identifier; TCIA, The Cancer Immunome Atlas; FIGO, The International Federation of Gynecology and Obstetrics; IPS, Immune Prognostic Score; ER, Estrogen receptor; POLEmut, POLE ultramutated; p53abn, p53 mutant; MMRd, Mismatch repair-deficient; NSMP, Non-specific molecular profile; TCGA, The Cancer Genome Atlas; DEPs, Differentially expressed proteins; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DO, Disease Ontology; FC, Fold Change; K-M, Kaplan-Meier; TMB, Tumor mutational burden; IHC, Immunohistochemical; FFPE, Formalin-fixed paraffin-embedded; BSA, Bovine Serum Albumin; PBS, phosphate buffer saline; qRT-PCR, Quantitative real-time polymerase chain reaction; CCK8, Cell Counting Kit-8; LVSI, Lymphovascular space invasion; OS, Overall survival.

Data Sharing Statement

The datasets used and/or analyzed in the current study are available from the corresponding author Lihua Wang MD on reasonable request. Some datasets analyzed for this study can be found in the TCPA (https://www.tcpaportal.org/tcpa/) and TCGA (https://portal.gdc.cancer.gov/).

Ethics Approval

The study was approved by the medical research ethics committee of the International Peace Maternal and Child Health Hospital (the ethical approval ID is [2015] No.2 and date of approval is April 19, 2016). The study complies with the Declaration of Helsinki.

Consent to Participate

The patients/participants provided written consent for participation in this study.

Acknowledgments

We thank the TCGA, TCPA database for providing RNA-seq expression profile data, clinical information and protein expression data of type I EC.

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; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the Shanghai Shenkang Three-year Action Plan for Promoting Clinical Skills and Clinical Innovation in Municipal Hospitals (SHDC2020CR5003-002) and the Project of Medical Engineering Cross Research Fund for "Star of Jiaotong University" of Shanghai Jiaotong University (YG2019QNA07).

Disclosure

The authors declare that have no competing interests in this work.

References

1. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2021. *Ca Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21708
2. Ueda SM, Kapp DS, Cheung MK, et al. Trends in demographic and clinical characteristics in women diagnosed with corpus cancer and their potential impact on the increasing number of deaths. *Am J Clin Exp Obstet Gynecol*. 2008;198(2):218.e1–218.e6. doi:10.1016/j.ajog.2007.08.075
3. Braun MM, Overbeek-Wager EA, Grumbo RJ. Diagnosis and management of endometrial cancer. *Am Family Phys*. 2016;93(6):468–474.
4. Li X, Zheng S, Chen S, Qin F, Lau S, Chen Q. Trends in gynaecological cancers in the largest obstetrics and gynaecology hospital in China from 2003 to 2013. *Tumor Biol*. 2015;36(7):4961–4966. doi:10.1007/s13277-015-3143-6
5. Lortet-Tieulent J, Ferlay J, Bray F, Jemal A. International patterns and trends in endometrial cancer incidence, 1978–2013. *JNCI J National Cancer Inst*. 2018;110(4):354–361. doi:10.1093/jnci/djx214
6. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. *Lancet Oncol*. 2014;15(7):e268–e278. doi:10.1016/s1470-2045(13)70591-6
7. Huvila J, Pors J, Thompson EF, Gilks CB. Endometrial carcinoma: molecular subtypes, precursors and the role of pathology in early diagnosis. *J Pathol*. 2021;253(4):355–365. doi:10.1002/path.5608
8. Asaka S, Liu Y, Z-c Y, et al. ARID1A regulates progesterone receptor expression in early endometrial endometrioid carcinoma pathogenesis. *Mod Pathol*. 2023;36(2):100045. doi:10.1016/j.modpat.2022.100045
9. Clement PB, Young RH. Non-endometrioid carcinomas of the uterine corpus: a review of their pathology with emphasis on recent advances and problematic aspects. *Adv Anatomic Pathol*. 2004;11(3):117–142. doi:10.1097/00125480-200405000-00001
10. Abu-Rustum N, Yashar C, Arend R, et al. Uterine neoplasms, version 1.2023, NCCN clinical practice guidelines in oncology. *J National Compr Cancer Network*. 2023;21(2):181–209. doi:10.6004/jnccn.2023.0006
11. He D, Wang X, Zhang Y, Zhao J, Han R, Dong Y. DNMT3A/3B overexpression might be correlated with poor patient survival, hypermethylation and low expression of ESR1/PGR in endometrioid carcinoma. *Chin Med J*. 2019;132(2):161–170. doi:10.1097/cm9.0000000000000054
12. Vermij L, Smit V, Nout R, Bosse T. Incorporation of molecular characteristics into endometrial cancer management. *Histopathology*. 2019;76(1):52–63. doi:10.1111/his.14015
13. Levine DA. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67–73. doi:10.1038/nature12113
14. Köbel M, Atenafu EG, Rambau PF, et al. Progesterone receptor expression is associated with longer overall survival within high-grade histotypes of endometrial carcinoma: a Canadian high risk endometrial cancer consortium (CHREC) study. *Gynecologic Oncol*. 2016;141(3):559–563. doi:10.1016/j.ygyno.2016.04.008
15. Trovik J, Wik E, Werner HMJ, et al. Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial. *Eur J Cancer*. 2013;49(16):3431–3441. doi:10.1016/j.ejca.2013.06.016
16. Kreizman-Shefer H, Pricop J, Goldman S, et al. Distribution of estrogen and progesterone receptors isoforms in endometrial cancer. *Diagn Pathol*. 2014;9:1–8. doi:10.1186/1746-1596-9-77
17. Reid-Nicholson M, Iyengar P, Hummer AJ, Linkov I, Asher M, Soslow RA. Immunophenotypic diversity of endometrial adenocarcinomas: implications for differential diagnosis. *Mod Pathol*. 2006;19(8):1091–1100. doi:10.1038/modpathol.3800620
18. Gullo G, Etrusco A, Cucinella G, et al. Fertility-sparing approach in women affected by stage I and low-grade endometrial carcinoma: an updated overview. *Int J Mol Sci*. 2021;22(21):11825. doi:10.3390/ijms222111825
19. Fujiwara S, Takeo N, Otani Y, et al. Epiplakin, a novel member of the plakin family originally identified as a 450-kDa human epidermal autoantigen. *J Biol Chem*. 2001;276(16):13340–13347. doi:10.1074/jbc.M011386200
20. Matsuo A, Yoshida T, Yasukawa T, Miki R, Kume K, Kume S. Epiplakin1 is expressed in the cholangiocyte lineage cells in normal liver and adult progenitor cells in injured liver. *Gene Expression Patterns*. 2011;11(3–4):255–262. doi:10.1016/j.gep.2011.01.001
21. Blagoev B, Kratchmarova I, Ong S-E, Nielsen M, Foster LJ, Mann M. A proteomics strategy to elucidate functional protein-protein interactions applied to EGF signaling. *Nature Biotechnol*. 2003;21(3):315–318. doi:10.1038/nbt790
22. Santin AD, Bellone S, Buza N, et al. Regression of Chemotherapy-Resistant Polymerase ϵ (POLE) ultra-mutated and MSH6 hyper-mutated endometrial tumors with nivolumab. *Clin Cancer Res*. 2016;22(23):5682–5687. doi:10.1158/1078-0432.Ccr-16-1031
23. Altman DG, McShane LM, Sauerbrei W, Taube SE. Reporting recommendations for tumor marker prognostic studies (REMARK): explanation and elaboration. *BMC Med*. 2012;10(1):1–39.

24. Guan J, Xie L, Luo X, et al. The prognostic significance of estrogen and progesterone receptors in grade I and II endometrioid endometrial adenocarcinoma: hormone receptors in risk stratification. *J Gynecol Oncol*. 2019;30(1). doi:10.3802/jgo.2019.30.e13
25. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47–e47. doi:10.1093/nar/gkv007
26. Kolde R, Kolde MR. Package "pheatmap". *R Package*. 2015;1(7):790.
27. Shen T, Lu Y, Zhang Q. High squalene epoxidase in tumors predicts worse survival in patients with hepatocellular carcinoma: integrated bioinformatic analysis on NAFLD and HCC. *Cancer Control*. 2020;27(1):107327482091466. doi:10.1177/1073274820914663
28. Bland JM, Altman DG. Survival probabilities (the Kaplan-Meier method). *BMJ*. 1998;317(7172):1572–1580. doi:10.1136/bmj.317.7172.1572
29. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling tumor infiltrating immune cells with CIBERSORT. In: *Cancer Systems Biology: Methods and Protocols*. Springer; 2018:243–259.
30. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nature Methods*. 2015;12(5):453–457. doi:10.1038/nmeth.3337
31. Miao YR, Zhang Q, Lei Q, et al. ImmuCellAI: a unique method for comprehensive T-cell subsets abundance prediction and its application in cancer immunotherapy. *Adv. Sci.* 2020;7(7):1902880. doi:10.1002/advs.201902880
32. Charoentong P, Finotello F, Angelova M, et al. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep*. 2017;18(1):248–262. doi:10.1016/j.celrep.2016.12.019
33. Kato Y, Peled N, Wynes MW, et al. Novel epidermal growth factor receptor mutation-specific antibodies for non-small cell lung cancer: immunohistochemistry as a possible screening method for epidermal growth factor receptor mutations. *J Thorac Oncol*. 2010;5(10):1551–1558. doi:10.1097/JTO.0b013e3181e9da60
34. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. *Ann Oncol*. 2016;27(1):16–41. doi:10.1093/annonc/mdv484
35. Yoshida T, Shiraki N, Baba H, et al. Expression patterns of epiplakin1 in pancreas, pancreatic cancer and regenerating pancreas. *Genes Cells*. 2008;13(7):667–678. doi:10.1111/j.1365-2443.2008.01196.x
36. Ma D, Pan Z, Chang Q, et al. KLF5-mediated Eppk1 expression promotes cell proliferation in cervical cancer via the p38 signaling pathway. *BMC Cancer*. 2021;21(1):1–10. doi:10.1186/s12885-021-08040-y
37. Qiao Z, Dai C, Wang Z, et al. Epiplakin1 promotes the progression of esophageal squamous cell carcinoma by activating the PI3K-AKT signaling pathway. *Thoracic Cancer*. 2022;13(8):1117–1125. doi:10.1111/1759-7714.14366
38. Yang L, Huang W, Bai X, et al. Androgen dihydrotestosterone promotes bladder cancer cell proliferation and invasion via EPPK1-mediated MAPK/JUP signalling. *Cell Death Dis*. 2023;14(6):365. doi:10.1038/s41419-023-05882-1
39. Guo X, Hao Y, Kamilijiang M, et al. Potential predictive plasma biomarkers for cervical cancer by 2D-DIGE proteomics and Ingenuity Pathway Analysis. *Tumor Biol*. 2014;36(3):1711–1720. doi:10.1007/s13277-014-2772-5
40. Lee H, Kim K, Woo J, et al. Quantitative proteomic analysis identifies AHNAK (Neuroblast Differentiation-associated Protein AHNAK) as a novel candidate biomarker for bladder urothelial carcinoma diagnosis by liquid-based cytology. *Mol Cell Proteomics*. 2018;17(9):1788–1802. doi:10.1074/mcp.RA118.000562
41. Meng Y, Yang Y, Zhang Y, Yang X, Li X, Hu C. The role of an immune signature for prognosis and immunotherapy response in endometrial cancer. *Am J Transl Res*. 2021;13(2):532.