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# Expression and clinical significance of PD-1 in UCEC and its impact on tumor

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**ABSTRACT**

Programmed death 1 (PD-1) plays an important role in the immune escape, occurrence and development of tumors by inhibiting the function of immune cells. However, its prognostic value in uterine corpus endometrial carcinoma (UCEC) and its impact on the tumor microenvironment remain to be further explored. Transcriptional datasets were retrieved from the GEPIA, TIMER and TCGA databases. "edgeR" package was used for the identification of differentially expressed genes (DEGs) between two groups of patients (PD1-high and PD1-low group). Gene set enrichment analysis (GSEA) was performed to identify underlying pathways between betweenPD1-high and PD1-low groups functioning in UCEC. Gene Correlation Analysis was used to further confirm the associations of PD1 expression with T-cell-related genes. Cytoscape software was used to identify hub genes in DEGs. Kaplan-Meier analysis was performed to validate the prognostic value of hub genes. Mutational characteristics of UCEC patients according to PD1 levels were depicted and analyzed. We found that the transcriptional expression of the PD1 gene in UCEC tumor tissues markedly increased in cohorts from the GEPIA and TCGA databases. PD1 expression was negatively correlated with gene signatures associated with the T-cell receptor signaling pathway and primary immunodeficiency. GESA confirmed that PD1 expression was negatively correlated with gene signatures associated with the T-cell receptor signaling pathway. T-cell receptor complex-related genes, ZAP70, TRAC, CD3D, CD3E, CD8A, TRBC2, TRBV28 and CD247, showed significant positive associations with PD1 expression. The results of the Kaplan-Meier OS analysis indicated that PD1, TIGIT, FASLG, ICOS and TNFRSF9 are the protective factor for patients with UCEC. The top 5 genes of mutations in the low expression group, included PTEN (56%), PIK3CA (43%), TP53 (41%), TTN (39%), and ARID1A (37%). The genes with a higher proportion of mutations in the PD1-high group are PTEN (67%), TTN (62%), PIK3CA (53%), ARID1A (52%), and MUC16 (12%). The prognosis of UCEC patients with PD1 overexpression phenotype is worse than that of the PD1 low group, which is due to the involvement of the PD1 gene in the T-cell receptor signaling pathway. This study provides a further theoretical basis and reference for targeted therapy against PD1.

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## Introduction

Uterine corpus endometrial carcinoma (UCEC) is a gynecologic malignancy of the female reproductive system and is the first common gynecological malignancy in developed countries in Europe and the United States. In 2018, about 380,000 new cases of and 90,000 deaths cases of UCEC were reported worldwide (1). The incidence of UCEC is on the rise worldwide especially in young women (2). Data show that in recent years, the mortality rate of UCEC in China exceeds the incidence rate (3). Irregular vaginal bleeding occurs in 90% of patients with UCEC, mainly after menopause. 70%-80% of patients with UCEC are confined to the uterus at the time of diagnosis. Comprehensive staging surgery is feasible, combined with pathology and risk factors, postoperative adjuvant radiotherapy and chemotherapy and other comprehensive treatment (4). Targeted therapy is a promising treatment. It's helpful to search markers that can indicate the clinical outcomes of UCEC and understand the course of disease for finding novel therapeutic targets.

Nowadays, the molecular mechanism of tumor development has been fully recognized. Targeted therapy and immunotherapy have become hot fields in the treatment of UCEC. In patients with UCEC, there are inhibitory immune checkpoints such as PD-1, PDL1, and CDLA4 expressed by tumor cells and immune cells, which inhibit the activation and function of T-cells in vivo, and enable tumor cells to obtain immune escape (5).

PD-1 is an important immunosuppressive receptor in the human body, which is mainly expressed on the surface of human T-cells, B cells and macrophages. When tumor invasion occurs, PD-L1 specifically binds to PD-1 on tumor-infiltrating T lymphocytes and activates the downstream pathway of PD-1/PD-L1 to inactivate the proliferation and differentiation of T-cells, which reduces the body's ability to clear tumor cells and leads to immune escape of tumor cells (6,7). As a star factor, the molecular mechanism of PD-1 has been widely studied in UCEC.

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However, there are few studies on its bioinformatics analysis.

It's a strategy to inhibit PD-1/PD-L1 signaling for the normalization of the dysregulated tumor microenvironment (8). PD-1/PD-L1 therapy has shown potent anti-tumor activity in a variety of cancers. The PD-L1 level is determined by multiple factors, which leads to different meanings of PD-L1 positivity and negativity (9,10). However, the PD-L1 protein level is the main criterion for selecting patients who are more likely to have a response to PD-1/PD-L1 therapy. PD-L1 positivity may be caused by the induction of immune response or oncogenic constitutive PD-L1 upregulation (10). Therefore, it is of great value for treatment selection and efficacy prediction to deeply understand PD-L1 expression.

This study investigated the Expression level of PD1 in UCEC and its relationship with the prognosis of UCEC patients and explored the relationship between PD1 expression and T-cell receptor complex-related genes and Somatic Mutations. The results identified the critical role of the PD1 gene in UCEC progression. This provides a further theoretical basis and reference for targeted therapy against PD1.

## Materials and Methods

### Data and information

TCGA (https://tcga-data.nci.nih.gov/tcga/) provided the RNA-sequencing and clinical data for the UCEC dataset. GEPIA analyzed UCEC clinical data and RNA-seq data from the TCGA project. The expression level of the PD1 gene in various cancers was identified in the TIMER database (https://cistrome.shinyapps.io/timer/).

### Differentially expressed gene analysis

The "edgeR" package was used for the identification of differentially expressed genes (DEGs) between two groups of patients (PD1-high and PD1-low group) according to the median counts of PD1 expression. The significant DEGs were selected according to log 2 (Fold change) and P-value with criteria: |log2(Fold change)|>1 and P-value < 0.05.

### Enrichment analysis

Gene set enrichment analysis (GSEA) was performed to identify underlying pathways between betweenPD1-high and PD1-low groups functioning in UCEC. The R package "org.Hs.eg.db" was used to convert the gene names of DEGs to gene ID. R "clusterProfiler" (Version 3.14.3) package was used to perform Gene Ontology (GO) function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for exploring the possible functions of these DEGs. The threshold P-value < 0.05 was used to identify significantly different GO terms and signal pathways. The enrichment results were visualized using the R package "enrichplot" and "ggplot2".

### Gene correlation analysis

To further confirm the associations of PD1 expression with T-cell-related genes, correlations between the PD1 gene and T-cell-related genes were calculated using the basic R function "cor.test". The function "ggcatterstats" included in "ggstatsplot" package was used to generate the dot plot.

### PPI network analysis and prognostic analysis

To detect the underlying associations among DEGs, the DEGs were uploaded to STRING to construct a PPI network. The result of STRING analysis was analyzed using Cytoscape software for visual analysis and hub gene identification.

In order to identify novel prognostic markers and validate their prognostic value, Kaplan-Meier analysis was performed to evaluate the overall survival (OS) of patients from the TCGA cohort.

### Somatic mutation analysis

The somatic mutation data of 530 cases among 583 UCEC patients were obtained from the TCGA-GDC database. According to the transcriptional levels of the PD-1 gene in the original cohort, the mutation profiles were cut into two groups (PD1-high and PD1-low groups). The mutation maps were drawn using the function "oncoplot" in the "maftools" package. The "mafCompare" function in the "maftools" package was used to evaluate the mutant genes with significantly different distributions.

## Results

### High expression of PD1 in UCEC samples predicts poor prognosis of PD1 in UCEC

Using RNA sequencing profiles from the GEPIA and TCGA databases, we found that the transcriptional expression of the PD1 gene in tumor tissues markedly increased in cohorts from the GEPIA and TCGA databases (all P < 0.05, Figures 1A and 1B). We preliminarily evaluated the transcript levels of PD1 in TCGA in tumors and normal tissues of multiple human cancer types. The results indicate that the PD1 expression was higher in uterine corpus endometrial carcinoma (UCEC), breast invasive carcinoma (BRCA), head and neck cancer (HNSC), stomach adenocarcinoma (STAD), lung adenocarcinoma (LUAD), and liver hepatocellular carcinoma (LIHC) compared to the normal tissues (Figure 1C). These results suggest that PD1 functions as an oncogene in the aforementioned tumors.

To investigate the influences of PD1 overexpression on clinical prognosis, the survival analysis was based on overall survival. The Kaplan-Meier survival analysis showed that high tumor PD1 expression was associated with less favorable UCEC survival.

### Screening of differentially expressed genes between PD1-high and PD1-low group

The median expression level was used to divide the patients from the UCEC cohort into low and high-expression subgroups in order to evaluate the correlation between PD1 levels and clinicopathological characteristics. TCGA's publicly accessible tumor database (www.tcga.org), including 548 UCEC tumor, were selected for this study. In the 548 tumor samples, analysis of differential expression between the PD1-high and PD1-low groups was conducted, with thresholds of $|\log2FC| \geq 1$and $P < 0.05$. A total of 549 differentially expressed genes were identified between the two groups, 13 (2.37%) were highly expressed in the PD1 high-expression group, and 536 (97.63%) were highly expressed in the PD1 low-expression group. Figures 2A and 2B show the volcano plot and heatmap of the diffe-

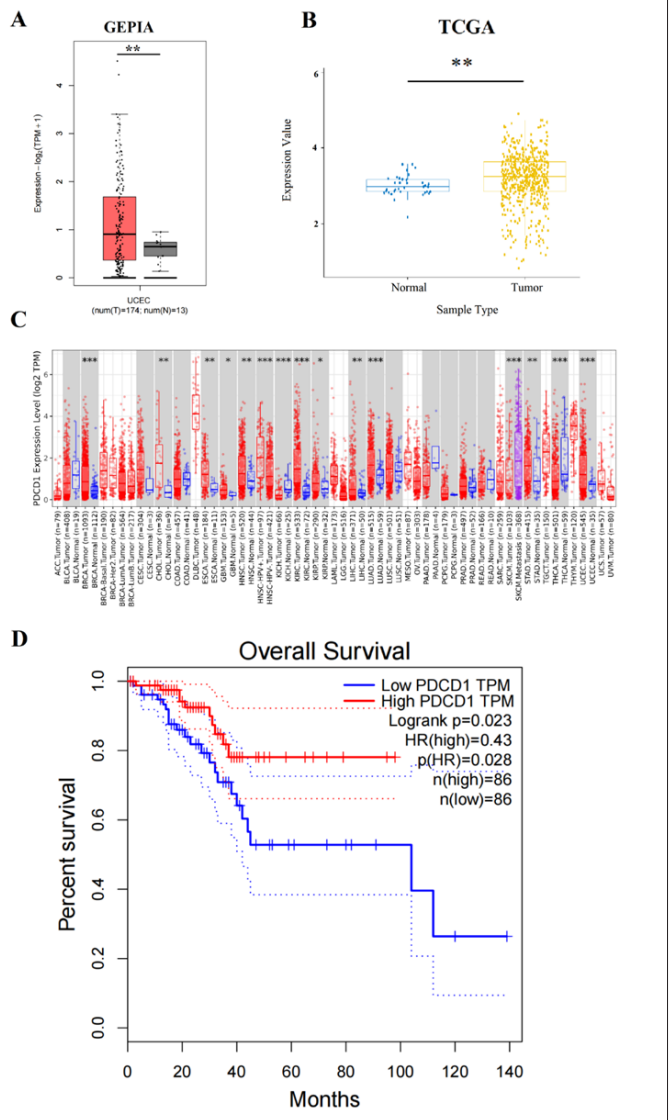


**Figure 1.** (A-B) The scatter diagram of PD1 expression between normal tissue and cancer tissue in GEPIA and TCGA. (C) The PD1 expression in pan-cancer according to the TIMER. (D) OS of the validation cohort stratified into PD1-high and PD1-low groups. *P < 0.05, ***P < 0.001.

rentially expressed genes between PD1-high and PD1-low groups.

## KEGG and GO enrichment analyses

To elucidate the potential function of PD1 in UCEC progression, the differentially expressed genes between PD1-high and PD1-low groups were enriched using KEGG or GO enrichment analyses.

Based on the GO enrichment analysis of biological processes, 10 groups were enriched, including positive regulation of leukocyte activation, leukocyte-mediated immunity and activation of the immune response. These biological processes were related to immune response. Cell components and molecular functions of these DEGs were also mainly about the preparation for the processes of immune receptor activity, immunoglobulin complex and T-cell receptor complex (Figure 3A). KEGG enrichment analysis showed that the DEGs were primarily enriched in the leukocyte transendothelial migration (Figure 3B).

To determine the signaling pathways significantly related to PD1 gene mRNA levels, various signaling pathways were analysed using gene set enrichment analysis (GSEA).

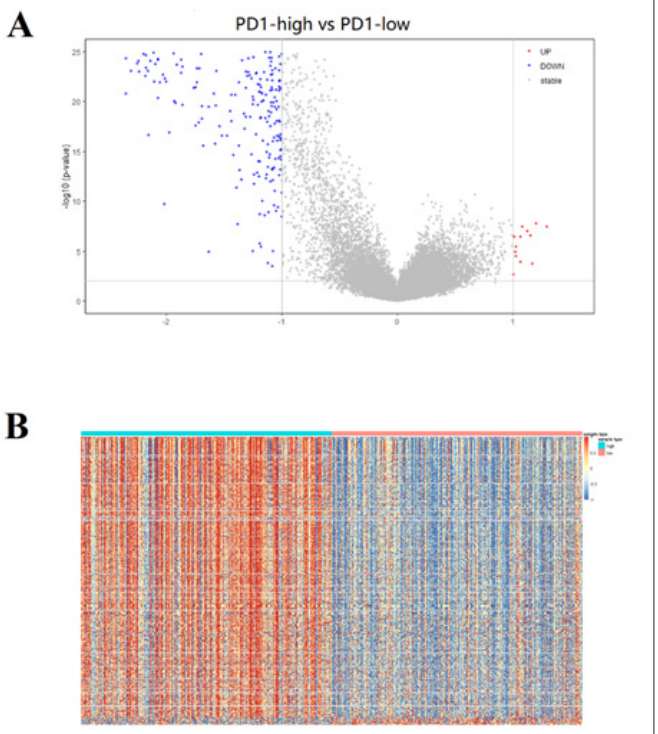


**Figure 2.** (A)Volcano plots displaying significantly differentially expressed genes in PD1-high and PD1-low groups. Red dots represent the upregulated genes and blue dots denote the downregulated genes, with thresholds of |log2FC| ≥ 1 and adjusted P < 0.05. (B) Heatmap displaying the expressions of the 549 DEGs PD1-high and PD1-low groups. Red bricks indicate the more highly expressed DEGs and blue bricks indicate lower expression.

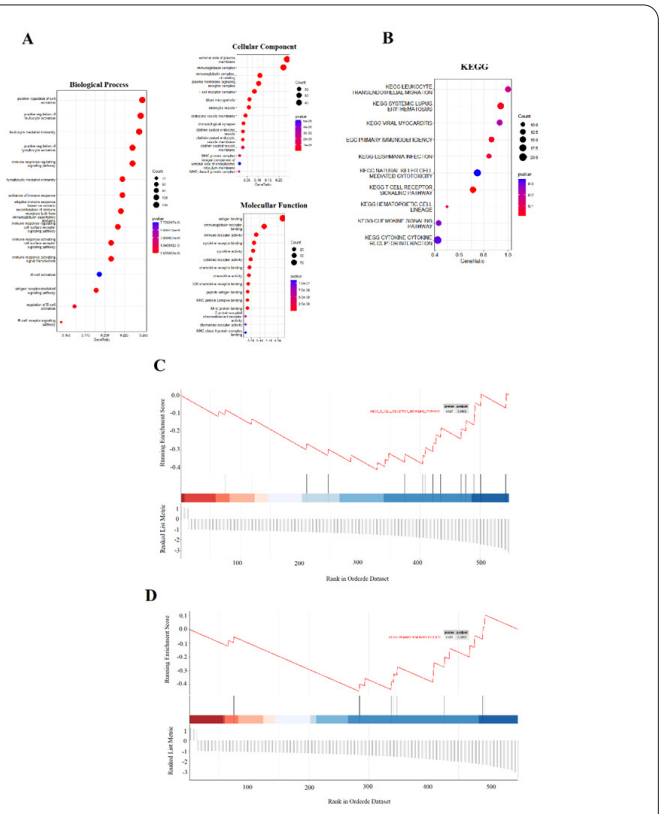


**Figure 3.** (A-B)The result of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses for the DEGs. (C-D) Gene set enrichment analysis (GSEA) prompts PD1 to be negatively related to T cell receptor signaling, and primary immunodeficiency.

GSEA analysis was also employed to pinpoint the signaling pathways markedly associated with the mRNA levels of the PD1 gene (PD1-high vs. PD1-low). GESA confirmed that PD1 expression was negatively correlated with gene signatures associated with the T-cell receptor signaling pathway and primary immunodeficiency.

**Analysis of PD1 co-expressed genes**

The co-expression analysis was performed to determine the genes correlated with PD1 expression in the UCEC cohort. The results show that T-cell receptor complex-related genes, ZAP70, TRAC, CD3D, CD3E, CD8A, TRBC2, TRBV28 and CD247, showed significant positive associations with PD1 expression (all $R > 0.8$, $p < 0.05$, Figure 4).

**Prognostic significance of Hub genes**

To explore the interaction between each DEGs, all DEG were submitted to the STING database, which is known for PPI. The PPI network was established and visualized using Cytoscape 3.8.1. After removing the isolated DEGs, a PPI network of DEGs with 291 nodes and 5103 edges was obtained (Figure 5).

According to the scores calculated using the MCC algorithm of cytoHubba, ten genes consider as the hub genes, including FASLG, TIGIT, PDCD1LG2, KLRB1, ICOS, CCL4L1, HAVCR2, NCR1, TNFRSF9, PD1. We further evaluated the prognostic significance of PD1 and the hubs genes in patients. The results of the Kaplan-Meier OS analysis indicated that PD1, TIGIT, FASLG, ICOS and TNFRSF9 are the protective factor for patients with UCEC (Figure 6).

**Relationship between somatic mutations and PD1 expression in UCEC**

The mutation profiles from the UCEC cohort in the TCGA database were collected to explore whether the distribution of mutations in the UCEC cohort was affected by PD1 gene expression. The mutation map of patients in two groups (PD1-high vs. PD1-low group) was profiled. The results showed that the top 5 genes of mutations in the low expression group, including PTEN (56%), PIK3CA (43%), TP53 (41%), TTN (39%), ARID1A (37%) (Figure 7B). The genes with a higher proportion of mutations in the PD1-high group are PTEN (67%), TTN (62%), PIK3CA (53%), ARID1A (52%), and MUC16 (12%) (Figure 7A).

We noted that patients in the PD1-high and PD1-low groups showed the highest rate of PTEN mutation, but the difference was not statistically significant (Figure 7D). In addition, the patients in the PD1-high and PD1-low groups showed a low rate of HMCN1 mutation, but the difference

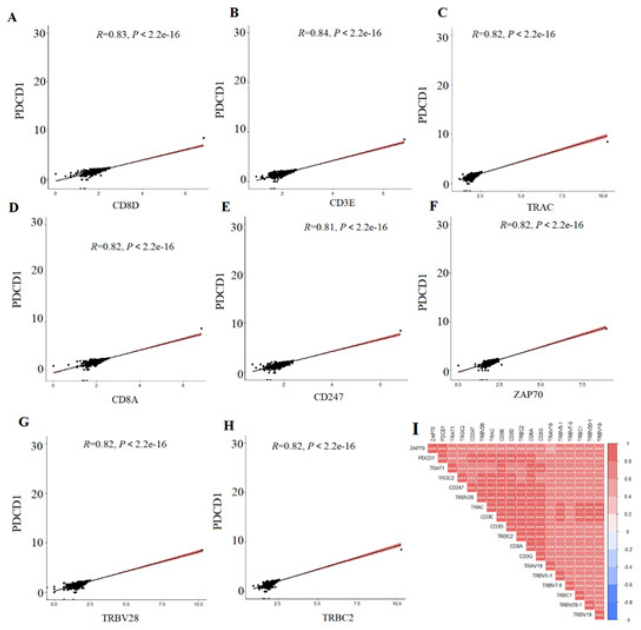


**Figure 4.** Correlation between T-cell receptor complex-related genes and PD1.

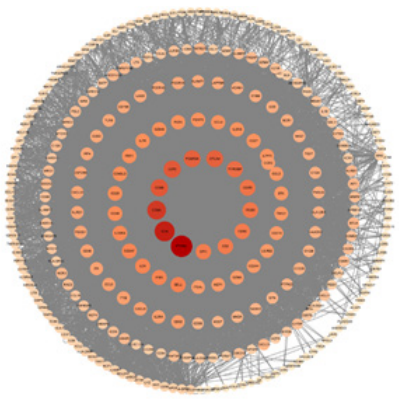

**Figure 4.** Visualization of the interaction network of genes strongly associated with PD1 by Cytoscape.

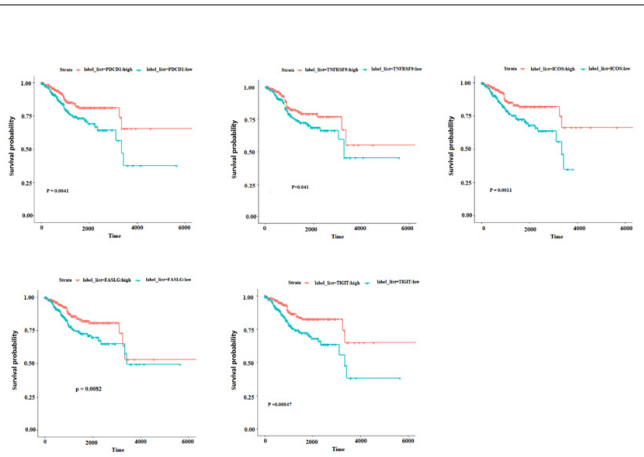


**Figure 6.** Prognostic Significance of Hub genes. Kaplan-Meier overall survival of hub genes.

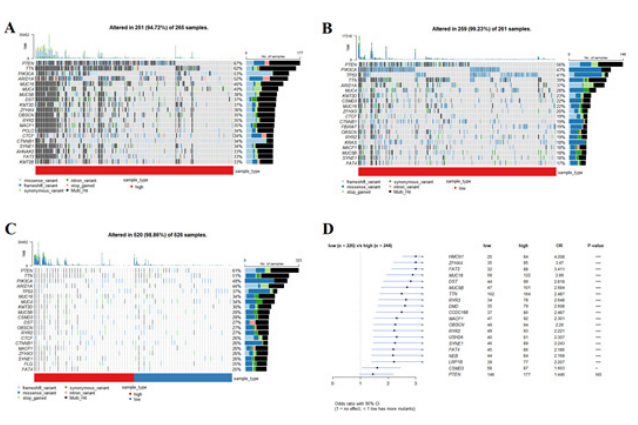


**Figure 7.** Relationship between somatic mutations and PD1 expression in UCEC. (A) Somatic mutations in PD1-high and PD1-low expression groups. (B) Comparison of mutations between the high expression group and low expression group of PD1.

was the largest and statistically significant (Figure 7D). The results showed that patients in the PD1-high group showed a higher rate of TTN, ZFHX4, and FAT3 mutation, and the difference was statistically significant. As shown in Figure 7D, the comparison of mutations in the PD1-high and PD1-low groups, all the top 20 genes had more mutations in the PD1-high group.

## Discussion

PD-1 is an important immune checkpoint (11). It inhibits the activation of T-cells and the production of cytokines through the action of its two ligands PD-L1 and PD-L2 and plays a crucial role in maintaining the body's peripheral tolerance (12). The immune checkpoint is a protective molecule in the human immune system, which acts like a brake to prevent inflammatory damage caused by excessive activation of T-cells (13). Tumor cells take advantage of this characteristic of the human immune system by over-expressing immune checkpoint molecules to inhibit the response of the human immune system and escape from human immune surveillance and killing, thereby promoting the growth of tumor cells (14). In recent years, the proposed and development of immunotherapy has set off a global anti-cancer upsurge, specially programmed death receptor 1 (PD-1) and programmed death receptor ligand 1 (PD-L1) inhibitors, which have been applied to a variety of tumor diseases and achieved good results (15).

To clarify the potential role of PD1 in UCEC, a systematic analysis of publicly available data was conducted. By exploring UCEC cohorts from TCGA, GEPIA and TIMER databases, we demonstrated that PD1 expression was significantly higher in tumor tissues than in non-tumor samples. The results of KEGG, GO and GSEA enrichment analysis showed that differentially expressed genes in tumor cells of the PD1-high and PD1-low groups had major differential enrichment in the activation of immune response and T-cell receptors. Kaplan-Meier OS analysis indicated that PD1, TIGIT, FASLG, ICOS and TNFRSF9 in hub genes are the protective factor for patients with UCEC. TIGIT (T-cell immunoglobulin and ITIM domain) is an inhibitory receptor expressed on lymphocytes, which has recently been considered the main new target of tumor immunotherapy (16). Because of its widespread expression in lymphocytes, TIGIT has become an important immune checkpoint, which is capable of inhibiting every step of the tumor immune cycle (17). FASLG plays an important role in the control of T-cell death by interacting with its cognate receptor FAS (18). ICOS (Inducible Co-Stimulator) acts as a T-cell-specific costimulatory receptor to promote T-cell responses to foreign antigens (19). TNFRSF9 (Tumor necrosis receptor superfamily) is considered to be a costimulatory receptor that can be induced by antigenic stimulation and is transiently expressed after T-cell activation (20). Taken together, these genes may be involved in the immune escape and progression of UCEC together with PD1, and are promising targets.

The correlation between PD1 expression and T-cell receptor complex-related genes was analyzed by co-expression. The results showed that T-cell receptor complex-related genes showed significant positive associations with PD1 expression. The T-cell immune response consists of the T-cell receptor first recognizing the peptide-bound MHC complex (pMHC) on the antigen-presenting cell (APC), and then the T-cell receptor transmits the antigen signal through its bound coreceptor (CD3) to the intracellular ITAM region of the ζ-subunit of CD3. In turn, a cascade of immune signaling pathways in T-cells is initiated to kill pathogenic infected cells or tumor cells (21).

PTEN (phosphatase and tensin homolog deleted on chromosome ten) had the highest mutation frequency in both PD1-high and PD1-low groups. PTEN is an important regulator of cell growth, survival, and metabolism. As a metabolic regulator, PTEN controls glucose and fatty acid metabolism (22). It indicated that PTEN may have an important relationship with the expression of PD1. The difference in HMCN1 mutation was the largest between PD1-high and PD1-low groups. Studies have shown that HMCN plays multiple roles in transient T-cell contacts required for cell migration and basement membrane invasion (23). However, there are few studies on the role of HMCN1 in tumorigenesis. This study found significant differences in the mutation frequency of HMCN1 between PD1-high and PD1-low groups, suggesting that HMCN1 may play a role in the development of UCEC by affecting the expression of PD1. TTN, ZFHX4 and FAT3 genes showed higher mutation rates in the PD1-high group. The abnormal expression of TTN-AS1 plays a crucial regulatory role in the occurrence and development of a variety of cancers (24-26). ZFHX4 has been identified as a key molecular regulator of tumor-initiating cells stem-cell--like function and Glioblastoma pathogenesis in multiple patient-derived samples (27). FAT atypical cadherin 3 (FAT3) is involved in planar cell polarity and tumor suppression (28).

In this study, differentially expressed genes (DEGs) were obtained by comparing PD1-high and PD1-low expression groups in the TCGA-UCEC group. These genes explained the effect of the PD1 gene on the biological behavior of UCEC tumor cells under KEGG and GO analysis. The survival status and mutation characteristics of patients were described and analyzed according to the PD1 level. The mutant morphology in the grouping case was also studied. This study provides a further theoretical basis and reference for targeted therapy against PD1.

### Ethical statement
The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was not involved in experiments of humans or animals.

### Consent for publication
Not applicable

### Availability of data and materials
The datasets generated during and/or analysed during the current study are available in the TCGA (https://www.cancer.gov), GEPIA (http://gepia.cancer-pku.cn/), and TIMER database (https://cistrome.shinyapps.io/timer/).

### Declaration of competing interest
The authors have no conflicts of interest to declare.

### Authors' contributions
Fengjuan Xing, Yan Yang, Wei Zheng: Substantial contributions to the conception or design of the work, the acqui-

sition, analysis, or interpretation of data for the work, and drafting the work or revising it critically for important intellectual content. All authors read and approved the final manuscript.

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