Prognostic Implications of PPL Expression in Ovarian Cancer

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Abstract

Periplakin (PPL) is a main member in plakin family, which plays important role in cellular adhesion complexes supporting and cytoskeletal integrity supplying. PPL was reported to be a potential biomarker candidate for several types of cancers. However, the biological functions and underlying mechanisms of PPL in ovarian cancer (OV) remain unclear. In the present study, we used GEPIA2, Human Protein Atlas, Oncomine, LinkedOmics, Kaplan-Meier Plotter, String, CytoHubba plug-in and TIMER to determine the associations among PPL expression, prognosis, and immune cell infiltration in OV. RT-qPCR and IHC analysis were conducted to validated the role of PPL in an independent OV cohort. Compared with the ovaries tissues from multiple datasets, levels of PPL mRNA and protein expression were obviously higher in OV tumors(P□0.05), and a poor survival in OV was found to be strongly correlated with high PPL expression (P<0.05). Moreover, the results were further validated by RT-qPCR and IHC analysis in an independent OV cohort. A gene-clinical nomogram was constructed, including PPL expression and clinical characterization in TCGA. Functional network analysis suggested that PPL regulates important pathways like Wnt signaling pathway, MAPK signaling pathway. Ten hub genes (LAMC2, PXN, LAMA3, LAMB3, LAMA5, ITGA3, TLN1, ACTN4, ACTN1, and ITGB4) were found to be positively associated with PPL. PPL expression was negatively correlated with infiltrating levels of CD4+ T cell macrophages, neutrophils, and dendritic cells. In conclusion, PPL may be an unfavorable prognostic biomarker candidate in OV, which was also correlated with immune infiltrating and might function in immunotherapy response.

1. Introduction

Ovarian cancer (OV) carries a very poor prognosis as a progressive and aggressive gynecological cancer (1). It ranks fifth in cancers leading to cancer-related mortality in female patients worldwide(2). The tumors have spread to peritoneum as well as adjacent organs when initially diagnosed in the vast majority of patients. And for patients at advanced stage, its five-year overall survival (OS) is only about 25–35%(3). There is no denying that the late diagnosis due to the lack specificity symptoms and primary or acquired chemotherapy resistance is the main reason for the unsatisfactory clinical outcome in OV(4). Thus, exploring the novel effective biomarkers for early diagnosis, prediction chemotherapy efficacy and prognosis might have a much bigger positive impact on the improvement of clinical outcome in OV.

Plakin is a big family of proteins, of which many members play important roles in cellular adhesion complexes supporting and cytoskeletal integrity supplying in different tissues(5). In cancer researches, plakins are an intriguing subject linked to many biological processes including cellular differentiation and migration, intracellular signaling, etc.(6). In plakin family, periplakin (PPL) was a main member identified in 1997, which was organized around desmosomes in differentiated keratinocytes, and primarily named the 195-kD protein periplakin(7). PPL is mainly localized in the desmosomes and inter-desmosomal plasma membranes of differentiated epidermal keratinocytes(8). In oncogenic threonine/serine protein kinase Akt/protein kinase B (PKB)-mediated signaling in human cancer cells, PPL can also function as a localization signal (9). Recent studies focusing on some molecular interactions suggests that PPL can

play a role of docking platform for various unrelated proteins. For urothelial carcinoma, PPL is a potential biomarker candidates(10). In human esophageal cancers, PPL was found to be significantly down-regulated, while PPL was scarcely expressed in its advanced-stage (11). What's more, for patients with urothelial bladder cancer, loss of PPL expression was observed to have certain association with cancer-specific survival and pathological stages(12). From above all, the possible role of PPL in development of various cancers has attracted more and more attentions. However, the underlying mechanisms of PPL expression in OV remain largely unknown.

To investigate the differential PPL transcriptional and proteomics expression and clarify the potential prognostic value in OV patients, PPL expression and mutations data of OV patients in The Cancer Genome Atlas (TCGA) and other public databases were investigated. Genomic alterations and functional networks related to PPL in OV patients were evaluated using multi-dimensional analysis, and its function in tumor immunity was explored. Results may provide some insights in potential targets and therapeutic strategies for OV patients.

2. Results

PPL Expression is up-regulated in OV patients' tissues

The PPL mRNA expression from GTEx projects and TCGA samples was compared using the GEPIA2 initially. The obvious differences were observed in fig 1a. (P□0.001). Then, the oncomine database was further used to analyze PPL mRNA levels in normal tissues and tumor tissues in a number of studies, which showed a higher PPL expression in OV tumors tissues than that in normal ovary (P□0.05, fig. 1b).Therefore, we surmised PPL up-regulation may serve as one of the tumor drivers in OV. However, the PPL expression profile may indicate the different roles of PPL in pan-cancer. A differential PPL mRNA expression was shown between normal tissues and tumor tissues across the different cancers (fig. 1c). Besides the differences between OV tissue and normal ovary in transcriptome level of PPL, the examination of the PPL protein level also suggested the consistent trend, showing the PPL protein level in OV tissues was higher compared with normal ovary tissues. Representative images of PPL staining in OV and normal ovary were shown in figure 1d from human protein atlas data, while figure 1e indicated the non-uniform differential of PPL protein level between the different types of cancers and normal tissues.

Elevation in PPL is associated with poor prognosis in OV patients

To investigate the prognostic implication of PPL in OV, Kaplan-Meier analysis was conducted based on the TCGA data. The patients were grouped according to the median value of PPL mRNA expression. The results displayed the group with high PPL expression had a significant shorter OS compared with the low group (OS: Cox P = 0.011, HR = 1.4, Fig. 2a). Furthermore, the similar results in OV were shown in the survival data depending on Kaplan-Meier plotter online tool (Fig. 2b). What's more, high PPL expression also showed a strong correlation with poor survival in pancreatic ductal adenocarcinoma, bladder cancer, and uterine corpus endometrial carcinoma (Fig. 2a-b). However, the inconsistent founding was shown in

adrenocortical carcinoma (ACC), sarcoma (SARC), and breast cancer, etc, indicating the low expression of PPL was correlated to poor survival.

Role of PPL in an independent OV cohort

The clinicopathological features of 42 recruited OV patients were listed in Table 1. The expression of PPL was tested in the 42 tumor samples and 10 normal ovaries, respectively. The mRNA expression and protein of PPL in OV tumors were both higher than those in normal ovary (P=0.001, P=0.001, fig. 3a-b, Table 2). Representative images of PPL protein staining were shown in figure 3b PPL protein was mainly expressed in cell membrane and cytoplasm. Among the 42 OV tumors, the positive expression rate of PPL was 90.47%. Among them, there were 17 cases with moderate staining, of which 7 cases were from the patient with FIGO Stage 2, 4 cases were from the patient with FIGO Stage 3 and 6 cases were from the patient with FIGO stage 4. The negative for PPL protein was only in 4 cases of OV.

Further, the 42 patients were divided into low and high group according to the median value of PPL mRNA expression. Kaplan-Meier analysis indicated that the PFS and OS of patients with PPL high group were obviously shorter compared with PPL low group (P = 0.008, P = 0.033, fig. 3c).

Evaluation and estimation of nomogram

Multivariable analysis for survival in TCGA was performed with PPL expression index included to detect if PPL plays a prognostic-associated role in OV patients. Results indicated that, when tumor residual size, grade, stage and age were included, a significant association between OS and PPL mRNA levels (P = 0.002, HR =1.3, 95%CI = 1.09-1.5, Fig.4a), suggesting that for OV patients PPL mRNA expression may be a potential independent prognostic biomarker figure 4b showed nomogram based on multivariate cox regression analysis. Depending on clinical information as well as genes, patients 3- and 5-year survival can be accurately predicted, and patients' prognosis can be visually predicted by nomograms. The model had a strong prediction power because of the predicted values were quite close to observed results of 3- or 5-year survival probabilities (fig. 4c).

PPL co-expression networks in OV

To gain a deep understanding of PPL biological functions in OV, the function fig 5A indicated the showed negative and positive correlation with PPL. The figure 5b presented top 50 significantly correlated genes (either negatively or positively). The figure 5c showed the STRING online database derived PPL network and its co-expression genes. A total of 502 PPL expression correlated essential genes were identified and listed in supplement table 1. By method MCC in CytoHubb, we found 10 hub genes (LAMC2, PXN, LAMA3, LAMB3, LAMA5, ITGA3, TLN1, ACTN4, ACTN1, ITGB4, Fig. 5d), of which the survival map of these ten genes in OV was shown in figure 5e.

Significant GSEA annotated geneontology_Biological_Process_no Redundant term indicated that PPL co-expressed genes mainly participate in Ras protein signal transduction, cell-cell signaling by wnt, cell cycle checkpoint, postreplication repair, DNA-templated transcription, termination, etc. KEGG pathway analysis

indicated the existence of enrichment in Wnt signaling pathway, MAPK signaling pathway, Hippo signaling pathway, EGFR tyrosine kinase inhibitor resistance, PI3K-Akt signaling pathway, etc. (fig. 5f).

PPL is correlated with immune infiltration level in OV

More importantly, we further assessed the underlying relationships of the mutants of PPL with immune infiltrates in OV microenvironment, a significant correlation was observed between PPL CNV and infiltrating levels of neutrophils and macrophages cells (fig. 6a). TIMER database results indicated a significant correlation between PPL expression and infiltrating levels of CD4+ T cell macrophages, neutrophils, and dendritic cells (fig.6b). Furthermore, Kaplan-Meier analysis results presented a correlation between poor survival outcomes in OV and lower infiltration levels of Dendritic cell (fig. 6c). The affection of PPL expression on various immune cells including neutrophil cell, dendritic cell, CD4+T cell, macrophage, CD8+T cell and B cell was evaluated using multivariable hazards models based on TIMER database (Table 3). CD4+T cell, Macrophage cell, and PPL expression were correlated with OS, based on Cox results . From above all, we surmised that PPL may have an impact on patients' survival through immune infiltration interaction in OV.

3. Discussion

According to public data, the PPL expression in OV tumor was demonstrated to be significantly higher than normal ovaries in this current study. The higher PPL expression is, the poorer the clinical outcomes of OV patients will be, and vice versa. After that, an independent OV cohort was carried out to further validate these results. Based on PPL expression, nomogram risk score in combination with clinical characterization can be used for predicting prognosis of OV patients, which is a potential OS predicting method. What's more, PPL expression also had a correlation with immune infiltration level in OV.

As a molecular bridge for cells, Plakin family members can link cell-cell junctions and intracellular cytoskeleton (6). Besides the PPL, Plakin families contain desmoplakin (DSP), envoplakin(EVPL), plectin(PLEC), and bullous pemphigoid antigen 1(HLA-DRB1). These members can function in different ways to create cytoskeleton elements connection and thus forming intercellular junction complexes. For example, plectin is such a most well-studied plakin, which can interact and function in signal transduction(13). According to the GEPIA2 online tool, we found that the expression of HLA-DRB1, PLEC, EVPL and DSP was significantly higher in OV tissues than normal ovary tissues (supplement fig 1). This is consistent with the PPL results in tumor and normal tissues. This suggests that members of the Plakin family may co-express and promote carcinogenesis during OV tumorigenesis and development. Although previous research found that PPL in a variety of tumors showed opposite conclusions, that is, the expression in the tumor tissues was significantly lower than that in the adjacent tissues, like advanced-stage of urothelial bladder cancer and human esophageal cancers. These inconsistent results may be related to the tumor tissue specificity.

Moreover, several research have reported an interaction with protein kinase B (PKB/c-Akt)1, suggesting PPL can relocate it to different cell compartments. Meanwhile during anti-apoptosis, PKB signaling may

also present an important function. Besides docking site, PPL has other roles to play, like shuttle of PKB delivery to different sub-cellular compartments after PKB was activated (9). We can find aberrant expression of AKT in many cancers including gastric, pancreatic, ovarian, lung, and breast carcinoma(14, 15). Based on the TCGA data in OV, the positive correlations between PPL and AKT1 expression were observed (supplement fig 2). A possible mechanism by which PPL participates in the occurrence of OV is through the regulation of AKT1 expression.

PPL function network was also conducted to explore the PPL co-expression and related signaling events, thus we can further reveal the underlying mechanisms of PPL in OV pathogenesis. Totally, we identified that 10 hub genes (LAMC2, PXN, LAMA3, LAMB3, LAMA5, ITGA3, TLN1, ACTN4, ACTN1, ITGB4) showed a positive association with PPL. Previously study presented a higher mRNA expression levels of laminin family members LAMC2, LAMB3 and LAMA5 in OV compared with normal ovary tissues. LAMC1 and LAMA5 may be prognostic factor and serve as important oncogenes in OV(16). LAMA3 was an unfavorable prognosis biomarker in OV(17). In ovarian clear-cell carcinogenesis, actinin-4 overexpression and genomic gain of ACTN4 may be the early molecular events(18). This supportive information enhances the understanding of the important role of PPL in OV. Possible molecular mechanisms of PPL involved in OV invasion and progression still need to be further explored by investigations in future. Furthermore, for the first time, the OV patients with higher PPL expression presenting poorer prognosis was observed from multiple cohorts and this result was validated in independent 42 OV patients. Thus, determination of PPL expression levels in surgical specimens of OV could aid in identifying and predicting the prognosis. What's more, using databases, the PPL prognostic landscape was visualized across all cancer types. Higher PPL expression also shown a correlation with poorer prognosis in uterine corpus endometrial carcinoma, pancreatic ductal adenocarcinoma, as well as bladder cancer.

OV immunotherapies have been attracting increasing interest based on the results that improved clinical outcomes have associations with tumor infiltrating lymphocytes (19, 20). Thus, for developing new immunotherapeutic strategies, a deeper insight into biological mechanisms and understanding of interaction between immunology and cancer are needed. In OV patients, PPL expression was negatively correlated with infiltrating levels of CD4+ T cell macrophages, neutrophils, and dendritic cells, which may provide power proof of the associations between PPL expression and immune markers. Also, to elucidate the important role of PPL in immunotherapy, more studies are needed in the future.

From above all, our research studied the PPL expression in OV and its value in prognosis. High PPL expression was proved to be a potential unfavorable biomarker for OV; what's more, multiple cohort studies also validated its prognostic value in pan-cancer. A potential correction between immune cells and PPL expression implies that PPL have the potential of crucial function in tumor immune microenvironment. The potential roles of PPL in OV are highlighted in this study. Therefore, future researches aiming at exploring underlying mechanism of PPL associated signaling pathways are required in further experiments.

4. Conclusion

In this study, we explored the prognostic implication of PPL in OV patients for the first time. Our data suggested PPL may be an unfavorable independent prognostic biomarker candidate in OV. Moreover, our results suggest that PPL co-expression genes in ovarian cancer may likely have far-reaching effects in pathway like Wnt signaling pathway, MAPK signaling pathway. PPL was also correlated with immune infiltrating and might play an important role in immunotherapy response.

5. Material And Methods

GEPIA2 analysis

Gene expression profiles from the Genotype-Tissue Expression (GTEx) projects and the Cancer Genome Atlas (TCGA) dataset were analyzed using the GEPIA 2 database (http://gepia2.cancer-pku.cn) (21). Normal and cancer tissues from 33 cancer types were analyzed for PPL expression using "Dot plot" function of GEPIA2. Log2(TPM + 1) scale was used to present gene expression.

Oncomine analysis

The Oncomine database (http://www.oncomine.org) includes 264 independent datasets, which further include 35 cancer types. These datasets support diverse analyses methods including meta-analysis, interactome analysis as well as molecular concepts analysis (22). Therefore, the Oncomine database was adopted to verify PPL expression. Expression level analyses were done by plotted in R using data acquired from Oncomine database.

The Human Protein Atlas (HPA)

The HPA (https://www.proteinatlas.org/) database contains proteomic and transcriptomic data from organs, tissues as well as cells. All these specimens are obtained from pathological or normal human tissues using immunohistochemistry (IHC) and RNA sequencing (RNA-Seq) analysis (23). HPA database (https://www.proteinatlas.org/pathology) was adopted in this study to obtain immunohistochemistry data of PPL expression in normal ovary and OV patient tissues.

Nomogram construction

For each of the potential risk factors that constructed the nomogram based on pre-processing PPL expression which was obtained from UCSC Xena, the Cox proportional hazard regression was performed to calculate the hazard ratio (HR), as well as the corresponding 95% confidence interval (CI). Nomogram and calibration plots were performed using P-value, HR, and 95% CI of each variable of forest plot obtained from ‘forestplot’ R package. ‘rms’ package from R. Nomogram can predict patient prognosis through integrating different prognostic factors to produce personalized clinical event probability. The calibration curves were used for assessing nomogram-predicted 3- and 5-year survival with observed 3- and 5-year survival.

4.5 Pathways Interaction Analysis Building and Protein Interaction Network (PPI)

In this study, the LinkedOmics database(24) (http://www.linkedomics.org/login.php) was adopted for PPL co-expression analysis based on Pearson's correlation coefficients. Heat maps and volcano plots were used to evaluate the results. LinkInterpreter of LinkedOmics was used to conduct analysis of GO_BP, KEGG pathways enrichment by the gene set enrichment analysis (GSEA). The rank criterion was P-value < 0.05 and 1000 simulations were performed. SRTING (v11.5, http://string-db.org/) (25) was used for the PPI information retrieval of PPL co-expression genes. A cutoff of 0.4 for minimum interaction score was set to obtain biological functions, with disconnected nodes hidden from network. After that, Cytoscape3.9.0(26) was applied to visualize the interaction network of these proteins, hub genes(filtering degree ≥10) were also acquired using CytoHubba plug-in.

TIMER database analysis

The Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) can be used to evaluate tumor-infiltrating immune cells in a diversity of cancer types (27). The TIMER includes over 10,000 samples from TCGA in diverse cancer types. The abundance of immune infiltrates was calculated using a partial deconvolution linear least square regression method. Based on "Survival" and "SCNA" module of TIMER database, the mutation types of PPL with immune infiltrates in OV patients were further evaluated.

Sampling of tissue specimens

Between Jan. 2018 and Jan. 2019, we collected the tissue samples from 42 OV patients when they received first surgery. Patient characteristics are listed in Table 1. The follow-up was regularly carried out for the following three years. Survival status of the OV patients was evaluated by overall survival (OS) and progression-free survival (PFS). 10 normal ovarian epithelial tissues were gathered from patients received adnexectomy due to the gynecological benign diseases concurrently. The Ethics Committee of Affiliated Xingtai People Hospital of Hebei Medial University reviewed and approved our study (2022 [05]). Written informed consent was provided by all patients.

The analysis of PPL mRNA and protein in an independent OV cohort

TRIzol reagent (Generay Biotech,China) was used to extract total RNA, based on manufacturers protocol. Using Revert-Aid First Strand cDNA Synthesis Kit (Thermo Scientific, U.S.A.), 500ng total RNA was used to synthesize cDNA. With GAPDH as housekeeping gene and primers bought from Sangon Biotech Co. Ltd. (Shanghai, China), QuantiNova TMSYBR® Green PCR Kit (Qiagen, Hilden, Germany) was applied for reverse transcription quantitative PCR(RT-qPCR). Custom primers for PPL (forward: TGCAGACCCGGAGCATCTCT reverse: CCTTCTGCAGGGTCACGTCC) was acquired from Sangon Biotech Co. Ltd. (Shanghai, China).

Rabbit momoclonal [ERP8296] to Periplakin (ab131269, Abcam, Cambridge, UK,) was applied to detect PPL protein in the tissue samples. The predicted location for PPL was considered in intracellular.

Specifically, the definition of strong, moderate, weak and negative staining was nuclear staining of >75% , 25%-75%, ≤25% and no nuclear staining of cells, respectively.

Statistical analysis

R software v3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) was applied for all statistical analysis. Pearson's chi-square test or Fisher's exact test was applied for analyzing qualitative variables. Quantitative variables analyses were performed using Wilcoxon rank-sum test (for unpaired samples). Kaplan-Meier analysis was adopted for survival analysis. Kruskal-Wallis test was carried out for normal multiple groups. If not specified above, *P values* < 0.05 were statistically significant.

Abbreviations

ACC adrenocortical carcinoma

CI confidence interval

DSP desmoplakin

EVPL envoplakin

HR hazard ratio

HLA-DRB1 bullous pemphigoid antigen 1

HPA Human Protein Atlas

IHC immunohistochemistry

GSEA gene set enrichment analysis

GTEx Genotype-Tissue Expression

OS overall survival

OV ovarian cancer

PLEC plectin

PFS progression-free survival

PKB protein kinase B

PPI Protein Interaction Network

PPL periplakin

RT-qPCR reverse transcription quantitative PCR

SARC sarcoma

TCGA The Cancer Genome Atlas

TIMER tumor Immune Estimation Resource

Declarations

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Bei-bei Zhao, Shao-bei Fan, Cai-fen Zhao, Yun-hong Kong, Rui-qing Tian, and Bao-ying Zhang. The first draft of the manuscript was written by Tian Hua and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The data sets generated and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Conflict of interest

All authors declare no conficts of interest.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the affiliated Xingtai People Hospital of Hebei Medial University (Date.2017.6/No.05).

References

1. Katsumata, N., Yasuda M., Isonishi S., Takahashi F., Michimae H., Kimura E., Aoki D., Jobo T., Kodama S., Terauchi F.; Long-term results of dose-dense paclitaxel and carboplatin versus conventional paclitaxel and carboplatin for treatment of advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer (JGOG 3016): a randomised, controlled, open-label trial. Lancet Oncol. 2013;14(10),1020-6.

2. Siegel, R. L., Miller K. D., Jemal A.; Cancer statistics, 2020. Ca A Cancer Journal for Clinicians. 2020;70(1).
3. Yang, S. P., Su H. L., Chen X. B., Hua L., Chen J. X., Hu M., Lei J., Wu S. G., Zhou J.; Long-Term Survival Among Histological Subtypes in Advanced Epithelial Ovarian Cancer: Population-Based Study Using the Surveillance, Epidemiology, and End Results Database. JMIR Public Health Surveill. 2021;7(11),e25976.
4. Friedlander, M., Trimble E., Tinker A., Alberts D., Avall-Lundqvist E., Brady M., Harter P., Pignata S., Pujade-Lauraine E., Sehouli J., Vergote I., Beale P., Bekkers R., Calvert P., Copeland L., Glasspool R., Gonzalez-Martin A., Katsaros D., Kim J. W., Miller B., Provencher D., Rubinstein L., Atri M., Zeimet A., Bacon M., Kitchener H., Stuart G. C.; Clinical trials in recurrent ovarian cancer. Int J Gynecol Cancer. 2011;21(4),771-5.
5. Fuchs, E.; Bridging cytoskeletal intersections. Genes Dev. 2001;15(1),1-14.
6. Sonnenberg, A., Liem R.; Plakins in development and disease. Exp Cell Res. 2007;313(10),2189-203.
7. Ruhrberg, C., Hajibagheri M. A., Parry D. A., Watt F. M.; Periplakin, a novel component of cornified envelopes and desmosomes that belongs to the plakin family and forms complexes with envoplakin. J Cell Biol. 1997;139(7),1835-49.
8. Simon, M., Green H.; Participation of membrane-associated proteins in the formation of the cross-linked envelope of the keratinocyte. Cell. 1984;36(4),827-34.
9. Heuvel, V. D., Pj A.; Binding of protein kinase B to the plakin family member periplakin. J Cell Sci. 2002;115(20),3957-66.
10. Okusa, H., Kodera Y., Oh-Ishi M., Minamida Y., Baba S.; Searching for new biomarkers of bladder cancer based on proteomic analysis. Journal of Electrophoresis. 2008;52(1),19-24.
11. Nishimori, T., Tomonaga T., Matsushita K., Oh-Ishi M., Kodera Y., Maeda T., Nomura F., Matsubara H., Shimada H., Ochiai T.; Proteomic analysis of primary esophageal squamous cell carcinoma reveals downregulation of a cell adhesion protein, periplakin. Proteomics. 2010;6(3),1011-8.
12. Masatsugu, IWAMURA, Tsuyoshi, TOMONAGA, Yuichi, SATO, Shin, EGAWA, Masaomi, IKEDA; Loss of periplakin expression is associated with pathological stage and cancerspecific survival in patients with urothelial carcinoma of the urinary bladder. Biomedical Research. 2014.
13. Lunter, P. C., Wiche G.; Direct binding of plectin to Fer kinase and negative regulation of its catalytic activity. Biochem Biophys Res Commun. 2002;296(4),904-10.
14. Mabuchi, S., Kuroda H., Takahashi R., Sasano T.; The PI3K/AKT/mTOR pathway as a therapeutic target in ovarian cancer. Gynecol Oncol. 2015;137(1),173-9.
15. Carpten, J. D., Faber A. L., Horn C., Donoho G. P., Briggs S. L., Robbins C. M., Hostetter G., Boguslawski S., Moses T. Y., Savage S.; A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007;448(7152),439-44.
16. Diao, B., Yang P.; Comprehensive Analysis of the Expression and Prognosis for Laminin Genes in Ovarian Cancer. Pathology & Oncology Research. 2021;27.

17. Tang, L., Wang P., Wang Q., Zhong L.; Correlation of LAMA3 with onset and prognosis of ovarian cancer. Oncol Lett. 2019;18(3),2813-8.
18. Yamamoto, S., Tsuda H., Honda K., Takano M., Tamai S., Imoto I., Inazawa J., Yamada T., Matsubara O.; ACTN4 gene amplification and actinin-4 protein overexpression drive tumour development and histological progression in a high-grade subset of ovarian clear-cell adenocarcinomas. Histopathology. 2012;60(7),1073-83.
19. Li, J., Wang J., Chen R., Bai Y., Lu X.; The prognostic value of tumor-infiltrating T lymphocytes in ovarian cancer. Oncotarget. 2017;8(9),15621-31.
20. Marth, C., Wieser V., Tsibulak I., Zeimet A. G.; Immunotherapy in ovarian cancer: fake news or the real deal? Int J Gynecol Cancer. 2019;29(1),201-11.
21. Tang, Z., Kang B., Li C., Chen T., Zhang Z.; GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nuclc Acids Research. 2019(W1),W1.
22. §, D. R. R., Kalyana-Sundaram S., Mahavisno V., Varambally R., Yu J., Briggs B. B., Barrette T. R., § M. J. A., § C. K.-B., § P. K.; Oncomine 3.0: Genes, Pathways, and Networks in a Collection of 18,000 Cancer Gene Expression Profiles. Neoplasia. 2007;9(2),166-80.
23. Ponten, Fredrik; A Tissue-Based Map of the Human Proteome. Scandinavian journal of immunology. 2015;81(5),329-.
24. Vasaikar, S. V., Straub P., Wang J., Zhang B.; LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46(D1),D956-D63.
25. Szklarczyk, D., Gable A. L., Lyon D., Junge A., Wyder S., Huerta-Cepas J., Simonovic M., Doncheva N. T., Morris J. H., Bork P., Jensen L. J., Mering C. V.; STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47(D1),D607-d13.
26. Shannon, P.; Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 2003;13(11),2498-504.
27. Li, T., Fan J., Wang B., Traugh N., Chen Q., Liu J. S., Li B., Liu X. S.; TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res. 2017;77(21),e108-e10.

Tables

Table 1 Clinical characteristics of ovarian cancer patients

Characteristics	**NO. patients (%)**
	All patients (n=42)
Age	
≤50	14(33.33)
50	28(66.67)
Pathology	
Serous	37(88.10)
Endometrioid	5(11.90)
FIGO stage	
-	18(42.86)
-	24(57.14)
Histological grade	
G1	10(23.81)
G2	9(21.43)
G3	23(54.76)

FIGO: International Federation of Gynecology and Obstetrics.

Table 2 PPL protein expression differences between the ovarian cancer tissues and the normal ovary tissues

PPL expression	**Ovarian cancer tissues n (%)**	**Normal ovary tissues n (%)**	***P*-value**
Negative	4 (9.52)	9 (90.00)	0.000
Weak	16 (38.10)	1 (10.00)	
Moderate	17(40.48)	0(00.00)	
Strong	5(11.90)	0(00.00)	

Table 3 Multivariable hazards models evaluate the impacts of PPL expression on overall survival in the presence of infiltrating levels of multiple immune cells

cell types	coef	HR	95%CI_l	95%CI_u	*P*.value	sig
B cell	-1.184	0.306	0.001	120.754	0.698	-
CD8+Tcell	-3.221	0.04	0.001	1.692	0.092	-
CD4+Tcell	-15.677	0	0	0	0	***
Macrophage	9.986	21710.213	100.968	4668131	0	***
Neutrophil	10.407	33105.021	5.36	204468000	0.019	*
Dendritic	-1.282	0.277	0.003	26.984	0.583	-
PPL	0.359	1.431	1.187	1.726	0	***

Note: Rsquare= 0.085 (max possible= 9.98e-01) Likelihood ratio test p= 1.84e-08 Wald test p= 2.01e-08 Score (logrank) test p= 2.11e-08.

Survival(OV)~variables is the formula of user-defined Cox's regression model based on TIMER database. The coefficient coef reads as a regression coefficient. HR gives you the hazard ratio, and its lower and upper 95% confidential interval are showed in 95%CI_l &

95%CI_u.

Figures

Figure 1

The expression PPL in ovarian cancer (OV). (a) The comparison of the transcriptional level of PPL expression between the 426 OV tissues and 88 normal tissues in TCGA cohort (*P<0.01). (b) The PPL mRNA levels in the Adib ovarian, Hendrix ovarian, and Yoshihara ovarian datasets, respectively. (c)The comparison of PPL expression in pan-cancer tumor tissues and normal tissues based on TCGA and GTEX database. (d) PPL protein is detected in OV tissues while not detected in normal tissues based on data from the human protein atlas. (e) The PPL protein expression in multiple cancer tissues and normal tissues using online database.

Figure 2

Kaplan-Meier survival curves for comparing the higher and lower expressions of PPL in multiple types cancer cohorts. (a) The survival curves from the GEPIA 2 online tool. OV (ovarian cancer) ACC (Adrenocortical carcinoma) LGG (Brain Lower Grade Glioma) SARC (Sarcoma). (b)The survival curves from the Kaplan-Meier Plotter online tool.

Figure 3

Validation of the role of PPL in an independent ovarian cancer (OV) cohort. (a) The comparison of PPL expression between OV tumor and normal ovary. (b) The comparison of PPL protein expression between OV tumor and normal ovary. (c) Kaplan-Meier survival curve for OV

patients with the PPL high and PPL low expression.

Figure 4

The association between PPL expression and ovarian cancer (OV) patients' survival outcome via bio-informatics analysis (a) Forest plots for multivariate Cox analysis. (b) Nomogram predicting 3-year and 5-year survival. (c) Calibration curves. Calibration curves for 3-year and 5-year cancer specific survival probability depict the calibration of each model. The ideal nomogram was represented by the dashed line; The performance of current nomogram was represented by the solid line.

Figure 5

PPL co-expression genes in ovarian cancer (OV). (a) The global PPL highly correlated genes identified by Pearson test in TCGA cohort (LinkedOmics). (b) Heat maps presenting the top 50 genes positively and negatively correlated with PPL in OV. Red displays positively correlated genes and blue displays negatively correlated genes. (c) A network of PPL and its co-expression genes was conducted visually using STRING in OV. (d) The hub genes (LAMC2, PXN, LAMA3, LAMB3, LAMA5, ITGA3, TLN1, ACTN4, ACTN1, and ITGB4) associated with PPL were identified in OV. (e) The survival map of hub genes in OV patients (GEPIA 2). (f)Significantly enriched GO annotations and KEGG pathways of PPL in OV.

Figure 6

Correlations of PPL expression with immune infiltration level in ovarian cancer (OV). (a)PPL CNV affects the infiltrating levels of macrophages and neutrophils in OV. (b)Immune infiltrates in correlation with PPL

in B cells, CD4+T cells, CD8+T cells, neutrophil, and dendritic cells of OV (TIMER) (c)Kaplan-Meier analysis shown that the lower infiltration levels of dendritic cells correlated with poorer survival outcomes in OV (P<0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- s1.png
- s2.png
- supplementtable1.pdf