Overexpressing PLOD Family Genes Predict Poor Prognosis In Pancreatic Cancer

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Research

Keywords: PLODs, Pancreatic cancer, prognosis, mutations, tumors infiltrate immune cells

Posted Date: September 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-895700/v1

Version of Record: A version of this preprint was published at International Journal of General Medicine on March 1st, 2022. See the published version at https://doi.org/10.2147/IJGM.S341332.

Abstract

Background: Pancreatic cancer is a common malignant tumor of digestive system. It has the characteristics of low early detection rate, large surgical trauma and high mortality. Multiple studies have shown that the PLOD family genes are closely associated with tumor progression and metastasis in many human cancers. However, The prognosis and biological function of PLOD family genes in PAAD have not been deeply discussed.

Methods: GEPIA,GEO,HPA,CCLE,Kaplan-Meier Plotter,cBioPortal, LinkedOmics, DAVID6.8, STRING and TIMER were used to determine the prognostic values and biological function of PLOD family members in pancreatic cancer.

Results: The mRNA and protein expression patterns of *PLOD* family members were noticeably upregulated in PAAD compared to normal tissue. In addition, PLOD family gene expression is also upregulated in pancreatic cancer cell lines.PLOD1 was significantly correlated with histological grade and pathological grade of pancreatic cancer. PLOD2 is related to histological grade.The high expression of PLOD1-2 was significantly correlated with the poor overall survival rate and relapse-free survival rate in patients with PAAD. In addition, PLODs has high sensitivity and specificity in distinguishing pancreatic cancer from normal tissues.Through the functional enrichment analysis of PLODs-related genes in pancreatic cancer, we found that PLODs was enriched in collagen fiber tissue structure, lysine degradation and collagen biosynthesis.Pathway analysis was confirmed PLODs regulates the proliferation, migration, and metastasis of pancreatic cancer through the RalGEF-Ral signaling pathway.The expression levels of *PLOD1–2* were positively correlated with the activities of tumor-infiltrating immune cells, including CD8+T cells, neutrophils, macrophages and dendritic cells.The expression level of PLOD3 was inversely correlated with the infiltration level of CD8+T cells.The expression levels of PLOD1 were higher in pancreatic cancers with TP53 mutations as compared to pancreatic cancers without mutations.PLOD2 expression levels were increased in KRAS mutant pancreatic cancer.However, the expression level of PLOD3 is elevated in SMAD4 wild-type pancreatic cancer.

Conclusion: The findings of this study could propose that individual PLOD genes or PLOD family genes as a whole could be potential prognostic biomarkers for PAAD.

Introduction

Pancreatic cancer is a highly malignant tumor of the digestive system with almost the same mortality as morbidity, with a 5-year total survival rate of only about 9% [1].Pancreatic cancer is the fourth largest cause of cancer-related death in America [1], while in China it is the sixth largest cause of cancer-related death [2]. By 2030, pancreatic cancer may be the second cause of death in a malignancy in America[3].The molecular mechanism of the occurrence and progression of pancreatic cancer remain largely unknown. The microenvironment of tumors plays a key role in pancreatic cancer. Abundant extracellular matrix components such as collagen and hyaluronic acid as well as matricellular proteins create a highly dynamic and hypovascular TME with multiple biochemical and physical interactions among the various cellular and acellular components that promote tumor progression and therapeutic resistance[4].Collagen is an important part of the ECM. Collagen-cell interactions are involved in maintaining normal tissue function and promoting cancer progression [5-6].Primary collagen-lysine, 2-oxyglutarate 5-doxygenase (PLOD) lysine hydroxylated collagen, prompting its cross-linking and deposition, and abnormal lysine hydroxylation promotes the development of the tumor [7].The PLOD family consists of three members, PLOD1/2/3, they are highly homologous and PLOD family members are the lysyl hydroxylase responsible for the lysyl hydroxylation of collagen[7-8].More studies have shown that overexpression of PLOD family genes plays a role in promoting tumor cell proliferation[9], migration[10] and invading [11] in human cancer.This study explores the expression of PLOD

family genes in pancreatic cancer, biological significance and relevance of prognosis in patients with pancreatic cancer.

Materials And Methods

GEPIA2 analysis

The GEPIA Database (http://gepia.cancer-pku.cn/) is an online dataset for the expression spectrum of PLOD family members obtained through pan cancer analysis and comparing the gene expression spectrum in cancer and paired normal tissues [39].To analyze the expression of gene members of the PLOD family in various cancers, data from the TCGA and genotype tissue expression (GTEx) databases were obtained for visualization of the expression spectrum.The data screening conditions are: (1) Tumor type: pancreatic cancer; (2)tissue comparison: pancreatic cancer tissue vs normal pancreatic tissue; (3)data type mRNA; (4) significance: P <0.01; (5) Differential expression level: more than 2 times;

GEO analysis

GEO Database (https://www.ncbi.nlm.nih.gov/geo/) GEO is an international public repository that can archive and distribute the microarray, second-generation sequencing, and other forms of high-throughput functional genomics data submitted freely by the research group [40].Download the gene expression spectrum dataset GSE16515[41],GSE15471[42]. GSE16515 and GSE15471 adopted the platform ([HG-U133_Plus_2]Affymetrix Human Genic U133Plus2.0 Array).GSE16515 contains data on gene expression microarray from 36 pancreatic and 16 parascancerous tissues. GSE15471 contains gene expression microarrays from 39 pancreatic and 39 parascancerous tissues. The original data is preprocessed using R language(limma package), with the probe transform according to the annotation information of the chip platform, obtain the expression matrix data of GSE16515 and GSE15471, and extract the expression value of PLOD1/2/3. The ROC model was used to diagnose pancreatic cancer.

Human Protein Atlas (HPA) Data Analysis

The HPA dataset (https://www.proteinatlas.org/) is an open access program that allows researchers to have free access to the data to explore human proteins in different tissues [43]. The HPA database confirmed the immunohistochemistry of PLOD in patients with pancreatic cancer. Depending on the dyeing intensity and the dyeing amount. The classification criteria for protein expression levels were as follows: negative, not detected; weak and <25%, not detected; weak combined with either 25–75% or 75%, low; moderate and <25%, low; moderate combined with either 25–75% or 75%, medium; strong and <25%, medium; and strong combined with either 25–75% or 75%, high.

Kaplan-Meier Plotter survival analysis

The Kaplan Meyer plotter database data sources include GEO, EGA and TCGA. The primary purpose of the tool is to discover and verify biomarkers, survival, and prognosis in cancer patients based on a meta-analysis [44].We study the relationship between PLOD1/2/3 in pancreatic cancer and total survival and recurrence-free survival rates using mRNA sequencing data from this database of generic pancreatic cancer analysis.

receiver operating characteristic curve（ROC）

RNAseq data in TPM format for TCGA and GTEx processed uniformly by the Toil process (Vivian J et al.,2017) using the UCSC XENA (https://xenabrowser.net/datapages/) database. The corresponding normal tissue data from TCGA for PAAD(pancreatic cancer) and GTEx were extracted. TPM data in TPM (transcripts per million reads) format and log2 conversion after sample expression comparison, using R package (R package: pROC package [1.17.0.1. version) (for analysis) | | ggplot2 package (version 3.3.3) (for visualization)).

CCLE analysis

The database contains in-group atlas deep analysis of thousands of cancer cell lines and the analysis of genetic mutations in more than 1000 CCLE databases, RNA, Cancer Cell Line Encyclopedia cell lines [45]. We analyzed the expression of PLOD1/2/3 in the pancreatic cancer cell line using the CCLE database.

LinkedOmics database analysis

The LinkedOmics Database [46] (http://www.linkedomics.org/login.php) is a Web-based platform for analyzing 32 cubes associated with TCGA cancer. LinkedOmics's LinkFinder module is used to study differential expression genes associated with PLOD1, 2 and 3 in the TCGA Pancreatic Cancer (PAAD) cohort (n=183). Statistical analysis results were performed using the Pearson correlation coefficient. We analyzed the expression of PLOD family genes in TP53, KRAS, SMAD4, CDKN2A wild type and mutant pancreatic cancer using LinkedOmics database.

cBioPortal data analysis

cBioPortal for Cancer Genomics is a comprehensive network resource that can visualize and analyze multidimensional cancer genomic data [47]. Evaluation of copy number variants (CNV), mutations, and gene types in pancreatic cancer based on the cBioPortal online instructions. The P value set to 0.05 is considered to be significantly different.

DAVID

In order to reveal from the LinkedOmics database analysis that the PLOD family gene has a positively correlated 121 genes and 237 negative correlated genes in pancreatic cancer, the function of PLOD in pancreatic cancer was explored using the DAVID database [48]. In this study, gene ontology (GO) and Kyoto Encyclopedia Gene and Genomics (KEGG) pathway enrichment analysis of the PLOD family members and their 121 positive and 237 negative correlated genes, respectively. GO is a biological process of network building and module analysis from molecular to organism levels, including molecular function (MF), biological processes (BP), and cellular components (CC). The critical values of the important GO term and the KEGG pathway are the error discovery rate (FDR) of <0.05. Gene enrichment GO terms and pathways are sorted by enrichment score (-log10 (P value)).

TIMER analysis

TIMER is the Internet platform resource for a comprehensive survey of the relationship between immune cells and multiple types of cancer [49]. TIMER applied algorithms to evaluate the abundance of tumor infiltrating immune cells from the gene expression spectrum. In this database, we analyze the correlation between PLODs expression and immune infiltration abundances and that of PLODS co-expressed genes in pancreatic cancer.

Construction of functional protein interaction networks

The search tool for retrieval the interaction gene (STRING) database aims to build a functional protein association network by integrating known and predicted protein-protein association data from a large number of organisms[50]. The STRING resources are available from the http://string-db.org/. We use a STRING database to establish functional protein-protein interaction networks between the PLOD family genes. We select the interactions associated with Homo sapiens and show a confidence score of > 0.9 for 30 interactions.

Results

Transcriptional level of PLODs in cancer

Firstly, we analyzed the expression levels of three PLOD genes in different kinds of human cancer using GEPIA2 database. Three PLOD genes showed a relatively up-regulated expression pattern in most of the cancer types(Figure1A,Figure1B and Figure1C). Secondly We use the GEPIA2 database to compare PLODs mRNA expression between PAAD and normal pancreatic tissue. (Figuer. 2A, Figure 2B and Figure 2C). In the GEO database.we compared the mRNA expression of PLODs between PAAD and normal pancreatic tissue., using the GSE16515 dataset and the GSE15471 dataset, respectively. PLODs expression was significantly upregulated in PAAD when compared to normal pancreatic tissue. (Figure. 2D, Figure 2E and Figure 2F are GSE16515 Figure. 2G, Figure 2H and Figure. 2I are GSE15471).

Transcription level of PLODs in the pancreatic cancer cell line

The expression distribution of PLODs genes in different cell lines of pancreatic cancer is shown in the CCLE database. The horizontal axis in the figure represents the expression of the gene, the longitudinal coordinates are different cell lines, the size of the circular dot represents the expression volume, and different colors also represent the expression volume.Figure 3A shows the high expression of PLOD1 in Panc02.13, PANC-1, and BxPC-3 in pancreatic cancer cancer cell lines. Figure 2B shows the high expression of PLOD2 in PANC-1, Panc02.13, and SU86.86 in pancreatic cancer cell lines. Figure 3C shows the high PLOD3 expression of Panc02.13, M I A P A C a-2, and PANC-1 in pancreatic cancer cell lines.

Protein expression levels of PLOD in the human protein map

To further study the expression of PLOD at the protein levels in pancreatic cancer, we further validate their expression levels using the Human Protein Atlas (HPA) database. PLOD1 is moderately expressed in pancreatic cancer tissue and is not expressed in normal pancreatic tissue (Figure 4A). PLOD2 and PLOD3 were highly expressed in pancreatic cancer and were not expressed in normal pancreatic tissue (Figure 4B, Figure 4C).

Prognostic value of PLODs in patients with PAAD

To explore the prognostic value of PLODs in PAAD patients, we analyzed the Kaplan-Meier Plotter database based on mRNA expression of individual members of the PLODs family. The OS curves of the three PLOD members are demonstrated in Figure5A, respectively.Notably, high transcriptional levels of PLOD1 (P= 0.033) and PLOD2 (P= 0.0062), were markedly associated with shorter OS in PAAD patients.The prognostic roles of differentially expressed PLOD members in the RFS of PAAD patients were also explored. It was found that high transcriptional levels of PLOD1 (P= 0.005) and PLOD2 (P=0.00086) were remarkably associated with shorter RFS in PAAD patients.

High expression levels of PLOD3 mRNA were not significantly associated with OS and RFS in patients with PAAD (as shown in Figure 5B). In predicting the outcome of Normal and Tumor, the predictive ability of variable PLOD family genes is more accurate.Based on the TCGA and GTEx datasets, the ROC curve shows area under PLOD1 curve (AUC) of 0.975, confidence interval (CI) of 0.957-0.993, area (AUC) of 0.873 under PLOD2 curve, confidence interval (CI) of 0.832-0.914, area (AUC) of 0.964 under PLOD3 curve, and confidence interval (CI) of 0.943-0.986 (shown in Figure 6). In short, the PLODs family genes have strong sensitivity and specificity in pancreatic cancer.

Baseline table of the relationship between PLOD family genes and clinicopathological features of pancreatic cancer

Expression of PLODS in pancreatic cancer with KRAS, TP53, CDKN2A, SMAD4 mutations

We analyzed the expression level of PLOD family genes in KRAS,TP53,CDKN2A and SMAD4 mutant pancreatic cancer.Compared with pancreatic cancer without mutation, the expression level of PLOD1 in TP53 mutant pancreatic cancer was higher(Figure D). The expression of PLOD2 was increased in KRAS mutant pancreatic carcinoma(Figure B). However, the expression level of PLOD3 in SMAD4 wild type pancreatic cancer is increased(Figure I).

Genetic variation analysis of the PAAD PLODs

The cBioPortal online tool is used to analyze genetic variations in members of the PLOD family in patients with PAAD. As shown in Figure 8A, 43 (29%) PAAD patients exhibited significant alterations in the three PLOD genes, including amplification, deep deletion, truncating mutation,missensemutation , and transcriptional upregulation.Specifically, the percentage changes in the genetic alterations of PLOD1, PLOD2, and PLOD3 among PAAD patients were 16, 10, and 10%, respectively (Figure 8B).

PLOD family member expression was associated with PAAD immune infiltration levels

To explore the immune microenvironment, the relationship of the levels of immune infiltration and the expression of PLODs in PAAD was analyzed by TIMER database. Results show that all members of the PLOD family were not correlated with tumor purity. The level of PLOD1/2 expression was significantly positively associated with the infiltration levels of CD8 + T cells, neutrophils, macrophages, and dendritic cells (Figure 8A,Figure 8B). PLOD3 mRNA expression was negatively associated with the infiltration level of CD8 + T cells (Figure 8C).

Analysis of the genes associated with the PLOD family in PAAD

To further validate the function of PLODs-related molecules in PAAD, we analyzed the mRNA sequencing data of 178 PAAD patients in TCGA using the functional module of the LinkedOmics database. As shown in the volcanic map,the PAAD samples had 3,340, 4,829 and 2,251 genes with significant positive correlation with PLOD1, PLOD2 and PLOD3) (pink and red dots, respectively. While 3338,3803, and 5205 genes showed a significant negative correlation with PLOD1, PLOD2 and PLOD3 in the PAAD sample, respectively (Fig. 10A,, 10B and 10C). To find the molecules associated with PLODs in pancreatic cancer, 121 genes were shown positively correlated with PLOD1, 2 and 3 (Figure 10D) and 237 were negatively correlated with PLOD1, 2, 3 (Figure 10E).We found that RALA is a concurrent regulatory molecule of PLODs in pancreatic cancer (Figure 11A-C).

GO enrichment and KEGG analysis of PLOD1/2/3

To further explore the biological function of these PLODs interaction genes in PAAD, we construct the GO and KEGG pathways using DAVID.Figure 12 Results of positive co-expression gene biological processes (BP) show that these genes are mainly involved in cell division, response to hypoxia, intercell adhesion, and chromosomal separation. For GO cell composition (CC) analysis, the term apparent enrichment is spindle, sticky spots, egg fissure.Significantly enriched molecular function (MF) terms include L-ascorbic acid binding, oxidoretase activity, protocollagen-lysine 5-dioxygenase activity, and microtubule-binding protein.KEGG pathway analysis revealed extracellular matrix – receptor interaction, focal adhesion, and enrichment of cancer pathways.Figure 13 Results of negative coexpression gene biological processes (BP) show that branched chain amino acids, cardiac conduction function regulation, blood pressure regulation, and drug metabolism. For GO cell composition (CC) analysis, the mitochondrial matrix, neuron ocellular cell membrane, intracellular, myogenic fibers. Significantly enriched molecular function (MF) terms include nucleic acid binding, catalytic activity, monooxygenase activity, and hydrolylase activity. KEGG pathway analysis showed the degradation of valine, leucine, iseucine, metabolic pathway, reabsorption of proximal tubulate bicarbonate, and gastric acid secretion.

In conclusion, the results show that PLODs is primarily involved in tumor-related regulatory mechanisms such as local adhesion, adhesion spots, oxidoretase activity, tumor pathways, and lysine degradation.

Functional protein interaction network of the PLOD family genes

We began with the STRING database looking for interacting protein networks of the PLOD family. The enclosure of the network includes 10 functional partners with the highest interactive confidence scores, namely COL5A2, COL5A1, COL1A1, COL1A2, COLGALT1, COL3A1, COL4A1, COL4A2, COL12A1 and COL2A1.The inner shell includes 20 other functional partners, all with interactive confidence scores higher than 0.9. These biological process functions are mainly involved in hydroxylysine biosynthesis processes, negative regulation of post-translational protein modification, collagen synthesis processes, peptidtide-lysine hydroxylation.Molecular functions include primary collagen-lysine 5-dioxygenase activity, extracellular matrix structural components given to tensile strength, and primary collagen galactosyltransferase activity. KEGG pathway analysis showed protein digestion and absorption, ECM-receptor interaction, and adhesion spot enrichment, and lysine degradation. In conclusion, the above results indicate that the PLOD family genes are primarily involved in regulating collagen metabolism and extracellular matrix composition.

Discussion

In our study, the PLOD family genes were highly expressed in pancreatic cancer.In all PAAD patients, high levels of expression of PLOD1 and PLOD2 were associated in shorter OS and RFS .Through receiver operating characteristic curve (ROC), we found that PLODs has high accuracy in predicting the outcome of Normal and Tumor. Moreover, the high expression of PLOD1 was significantly associated with clinical tumor T stage, histological classification, and pathological classification. High expression of PLOD2 was significantly associated with the histological classification.Through gene ontology and pathway enrichment analysis, PLOD family genes are mainly involved in the regulation of collagen metabolism and extracellular matrix composition. RalGEF-Ral signal pathway may be a regulatory module that mediates the effect of PLOD on PAAD.

Several studies found that the abnormal expression of PLOD1 is closely associated with multiple malignancies in humans, including stomach cancer, colorectal cancer, liver cancer, and osteosarcoma [12-15]. n addition, Yamada Y et al [16], indicated that miR-140-5p could directly bind to the 3'-UTR of PLOD1, causing PLOD1 knocking to

significantly inhibit the migration and invasion of T24 cells.Jiang H et al[15],found that PLOD1 regulated the proliferation and invasion of osteossarcoma cells through the Wnt/ β -catenin signaling pathway.

The role and mechanism of PLOD2 in breast cancer has been deeply explored, and the abnormal expression of PLOD2 in breast cancer is regulated by the hypoxia-inducing factor 1a (HIF-1a), increases the tumor hardness of breast cancer, and promotes the metastasis of tumor cells to the lymph nodes and lungs [17].In addition, the mechanism of the hypoxia-induced factor 1a (HIF-1a) that enhances PLOD2 expression is used to control the metastasis of sarcoma [18].In pancreatic cancer, increased expression of PLOD2 under hypoaerobic conditions promotes movement of pancreatic cancer cells and thus tumor progression [19].This study is consistent with our results.

In addition,PLOD3 can promote lung cancer metastasis by regulating STAT3 [20].PLOD3 is upregulated in gastric cancer, promoting the proliferation of gastric cancer cells and affecting the prognosis of patients with gastric cancer [21].

In conclusion, the PLOD family genes are involved in the tumorigenesis of multiple cancers and affect the patient prognosis. we propose that individual PLOD genes or PLOD family genes as a whole could be potential prognostic biomarkers for PAAD.

With numerous studies in the genomics of pancreatic cancer, high-frequency mutations in Kras, TP53, SMAD4 and CDKN2A have been confirmed in pancreatic cancer and are closely related to the occurrence and development of pancreatic cancer[22].Tumor suppressor genes inactivated in pancreatic cancer include TP53 (mutated in 75-90% PDAC cases), P16 / CDKN2A (mutated in 50-98% PDAC) and SMAD4 (mutated in 20-50% PDAC) [23].High-frequency mutated genomic changes seen in pancreatic cancer lead to significant genomic instability and may limit the effectiveness of treatment by leading to secondary or acquired chemical resistance, especially the effectiveness of targeted drugs [22]. We unexpectedly found that there were significant differences in expression between PLOD family genes and TP53 mutation, KRAS mutation and SMAD4 mutation in pancreatic cancer. The PLOD family genes have the potential to be a therapeutic target for these high-frequency mutated genomic pancreatic cancers.

Functional enrichment analysis in our study to understand the biological function of PLOD in PAAD showed that these genes are primarily involved in collagen synthesis processes, peptidtide-lysine hydroxylation. Molecular functions include primary collagen-lysine 5-dioxygenase activity, extracellular matrix structural components given to tensile strength, and primary collagen galactosyltransferase activity.The KEGG pathway is enriched in ECM-receptor interaction and adhesion spot enrichment, lysine degradation.In previous studies, we found that invasion of pancreatic cancer was closely related with ECM [24]. Collagen is one of the main components of the extracellular matrix [25].The hydroxylation of lysyl residues catalyzed by PLODS is a key step in collagen biosynthesis [26].Lower PLODS expression levels can cause harmful changes in the collagen deposition and tissue of the extracellular matrix, which in turn causes changes in the morphology and cytoskeleton of the attached cells [27].The increase in collagen deposition enhances the biological process of migration, invasion, and proliferation of tumor cells [28-29].We can see that the PLOD family gene regulates the deposition and maturity of collagen promotes the development of cancer.

Previous studies have confirmed that the heterogeneous immune characteristics of pancreatic cancer have significantly affected the survival of patients with pancreatic cancer [31]. Patients with pancreatic cancer with long-term survivors have higher numbers of CD8 + T cells, hemolytic CD8 + cells, regulatory T cells, mature dendritic cells, and macrophages [30].Thus we analyze the infiltration rates of various immune cells (B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells) with different levels of PLOD gene expression. We observed a correlation between both PLOD family genes and immune cell infiltration.In particular, PLOD1 and PLOD2 are closely related to CD8 + T, neutrophils, macrophages, and dendritic cells. Previous studies have found that neutrophil adhesion to fibronectin can stimulate the release of hydroxylysine into the extracellular environment ,but had no effect on the secretion of other amino acids by neutrophils [32]. Hydroxylysine is a product of PLOD. Abnormal expression of PLOD affects the morphology and cytoskeleton of attached cells [27].

In the pathway study we found that PLODS family genes were significantly associated with RalA. Overactivation of the RalA signaling pathway in multiple human malignancies and higher RalA activation in cancer stem cells than differentiated cancer cells [33].Inhibition of RalA by shRNA in pancreatic cancer reduced non-adherent growth in vitro and subcutaneous growth in vivo [34].In addition, RalA is a key determinant of integrin-dependent membrane raft transport and growth signal regulation, and RalA is preferentially activated in pancreatic cancer, playing a core role [35-36].KRAS mutations are present in more than 90% of human pancreas [37],KRAS mutations can cause persistent activation of RalA through the RalGEF-Ral signaling pathway, prompting the proliferation, migration and metastasis of pancreatic cancer cells [38].Since the enzymatic activity of PLOD1, 2 and 3 has great effects on the composition and structure of ECM and that RalA is positively coexpressed with PLODs in PAAD, we assume that PLODs cooperates with RalA and participates in the RalGEF-Ral signaling cascade to regulate PAAD cell proliferation and migration by changing ECM. However, further research is needed.

This is the first report on the prognostic value of PLOD family genes in patients with PAAD. Our study provides a complete report on the correlation between PLOD family gene expression and prognosis in pancreatic cancer, which makes this study more convincing.Of course, this study has limitations. Our prognostic study was mainly based on the PAAD patient cohort from the TCGA and GEO databases. Although laboratory experimental studies can be consistent with a database, more independent cohorts of PAAD patients are needed to confirm our findings.In addition, PLOD3 plays a role in many kinds of human malignant tumors, but in our study, PLOD3 is abnormally expressed in pancreatic cancer, but it has no significant correlation with the prognosis of patients with pancreatic cancer. This needs to be verified by more clinical data and basic experiments of pancreatic cancer patients, which is what we need to do next.

Conclusion

The combination of PLOD family genes may be an excellent index for prognosis evaluation and diagnosis of pancreatic cancer. And We predict that PLOD family genes may promote proliferation, migration and metastasis of pancreatic cancer through RalGEF-Ral signal pathway.

Abbreviations

CH1 collagen homology 1

CH2 collagen homology 2

CI confident interval

DAVID Database for Annotation, Visualization and Integrated Discovery

ECM extracellular matrix

FDR false discovery rate

RFS Relapse Free Survival

PAAD Pancreatic adenocarcinoma

GO gene ontology

HR hazard ratio

KEGG Kyoto Encyclopedia of Genes and Genomes

RAL Ras-related protein Ral-A

OS overall survival

PLOD procollagen-lysine, 2-oxoglutarate 5-dioxygenase

CDKN2A Cyclin-dependent kinase inhibitor 2A

SMAD4 Mothers against decapentaplegic homolog 4

GEO GENE EXPRESSION OMNIBUS

ROC Receiver operating characteristic curve

KRAS GTPase KRas

STRING Search Tool for the Retrieval of Interacting Genes

TCGA The Cancer Genome Atlas

TP53 EKC/KEOPS complex subunit TP53RK

TPM ranscripts per million reads

CCLE Cancer Cell Line Encyclopedia

TIMER Tumor Immune Estimation Resource

STAT3 Signal transducer and activator of transcription 3

TME The tumor microenvironment

Declarations

Ethics approval and consent to participate

As all the data were retrieved from the online databases, so it could be confirmed that all written informed consent had already been obtained.

Consent for publication

Not applicable.

Availability of data and materials

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Competing interests

The authors declare that they have no competing interests.

Funding

The author(s) received no specific funding for this work.

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Authors' contributions

JZ complete the bioinformatics analysis, designed research and wrote draft. .XF analyze the data.YT and SM conduct a statistical analysis.All authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank the study participants and their families.

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Table

Table 1 Baseline table of the clinicopathologic characteristics of the PLODs family gene.The high expression of PLOD1 was significantly associated with clinical tumor T staging (P=0.038), histological classification (P=0.049), and pathologic classification (P=0.013). The high expression of PLOD2 is associated with the histological classification (P=0.038). PLOD3 was not significantly associated with clinicopathologic features.

Figures

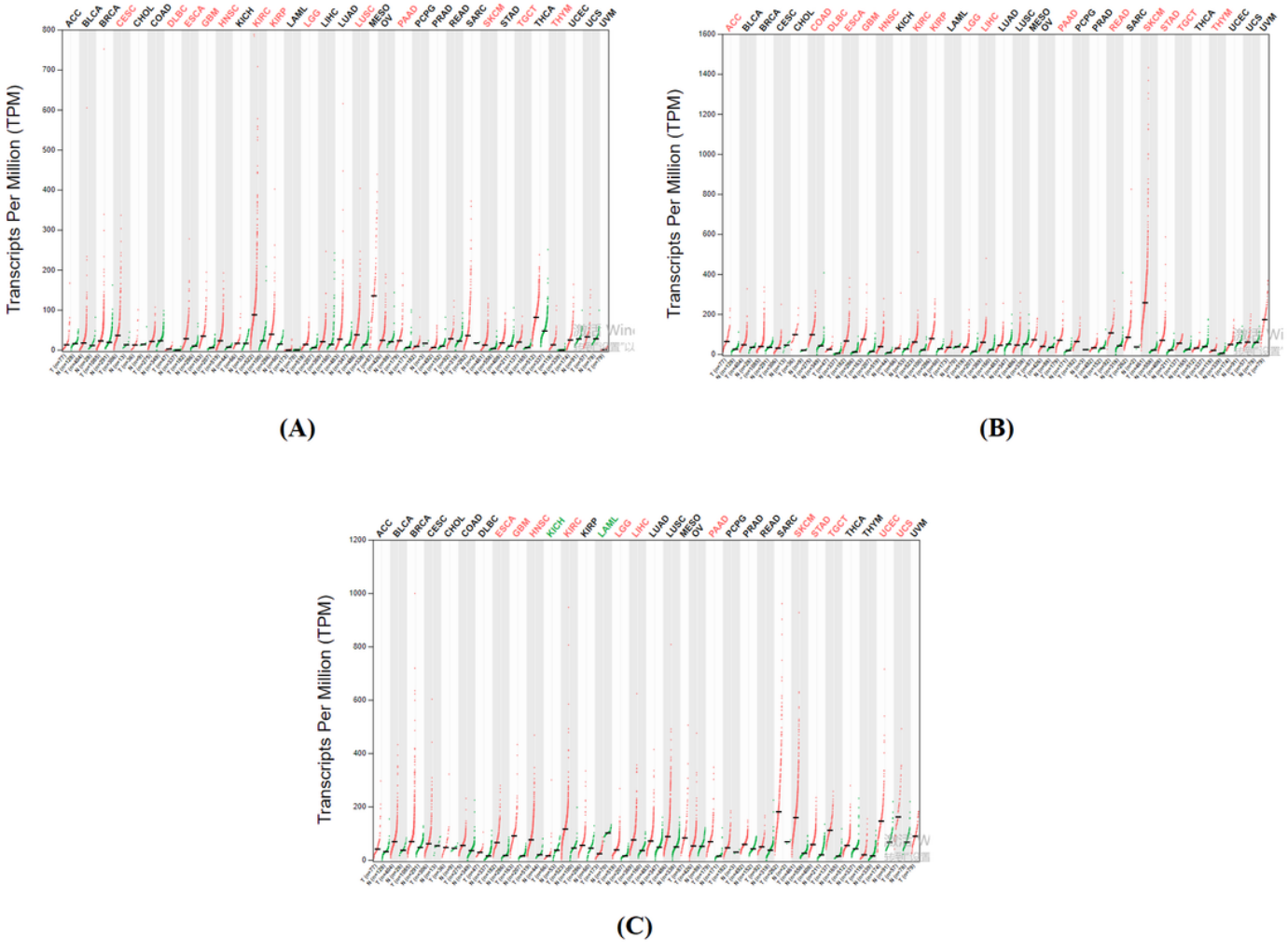

Figure 1

Expression of PLOD family members in different tumor tissues.(A) The transcript levels of PLOD1 in different types of cancer.Red, up-regulated; green, downregulated.(B) and (C) are the transcript levels of PLOD2, PLOD3 in different

Characteristic	Low expression of PLOD1	High expression of PLOD1	p	Low expression of PLOD2	High expression of PLOD2	p	High expression of PLOD3	p
n	89	89		89	89		89	
T stage, n (%)			**0.038**			0.215		0.334
T1	6 (3.4%)	1 (0.6%)		6 (3.4%)	1 (0.6%)		2 (1.1%)	
T2	16 (9.1%)	8 (4.5%)		12 (6.8%)	12 (6.8%)		9 (5.1%)	
T3	64 (36.4%)	78 (44.3%)		67 (38.1%)	75 (42.6%)		75 (42.6%)	
T4	2 (1.1%)	1 (0.6%)		2 (1.1%)	1 (0.6%)		1 (0.6%)	
N stage, n (%)			0.119			0.058		0.187
N0	30 (17.3%)	20 (11.6%)		31 (17.9%)	19 (11%)		29 (16.8%)	
N1	56 (32.4%)	67 (38.7%)		55 (31.8%)	68 (39.3%)		56 (32.4%)	
M stage, n (%)			1.000			1.000		0.360
M0	33 (39.3%)	46 (54.8%)		33 (39.3%)	46 (54.8%)		38 (45.2%)	
M1	2 (2.4%)	3 (3.6%)		2 (2.4%)	3 (3.6%)		4 (4.8%)	
Pathologic stage, n (%)			**0.049**			0.147		0.586
Stage I	16 (9.1%)	5 (2.9%)		15 (8.6%)	6 (3.4%)		10 (5.7%)	
Stage II	68 (38.9%)	78 (44.6%)		68 (38.9%)	78 (44.6%)		71 (40.6%)	
Stage III	2 (1.1%)	1 (0.6%)		2 (1.1%)	1 (0.6%)		1 (0.6%)	
Stage IV	2 (1.1%)	3 (1.7%)		2 (1.1%)	3 (1.7%)		4 (2.3%)	
Gender, n (%)			0.651			0.451		0.175
Female	38 (21.3%)	42 (23.6%)		37 (20.8%)	43 (24.2%)		35 (19.7%)	
Male	51 (28.7%)	47 (26.4%)		52 (29.2%)	46 (25.8%)		54 (30.3%)	
Age, n (%)			0.133			0.007		0.764
<=65	41 (23%)	52 (29.2%)		37 (20.8%)	56 (31.5%)		45 (25.3%)	
>65	48 (27%)	37 (20.8%)		52 (29.2%)	33 (18.5%)		44 (24.7%)	
Histologic grade, n (%)			**0.013**			**0.038**		0.277
G1	23 (13.1%)	8 (4.5%)		22 (12.5%)	9 (5.1%)		11 (6.2%)	
G2	41 (23.3%)	54 (30.7%)		41 (23.3%)	54 (30.7%)		49 (27.8%)	
G3	22 (12.5%)	26 (14.8%)		23 (13.1%)	25 (14.2%)		27 (15.3%)	
G4	1 (0.6%)	1 (0.6%)		1 (0.6%)	1 (0.6%)		1 (0.6%)	
Alcohol			0.447			0.128		0.312

history, n (%)								
No	30 (18.1%)	35 (21.1%)		28 (16.9%)	37 (22.3%)		30 (18.1%)	
Yes	54 (32.5%)	47 (28.3%)		57 (34.3%)	44 (26.5%)		56 (33.7%)	
History of diabetes, n (%)			0.700			0.516		0.916
No	51 (34.9%)	57 (39%)		54 (37%)	54 (37%)		57 (39%)	
Yes	20 (13.7%)	18 (12.3%)		22 (15.1%)	16 (11%)		19 (13%)	
History of chronic pancreatitis, n (%)			1.000			1.000		0.303
No	63 (44.7%)	65 (46.1%)		65 (46.1%)	63 (44.7%)		64 (45.4%)	
Yes	6 (4.3%)	7 (5%)		7 (5%)	6 (4.3%)		9 (6.4%)	
Age, meidan (IQR)	67 (60, 74)	64 (55, 72)	0.038	69 (61, 74)	63 (55, 71)	0.003	64.67 ± 11.23	0.928

types of cancer, respectively.

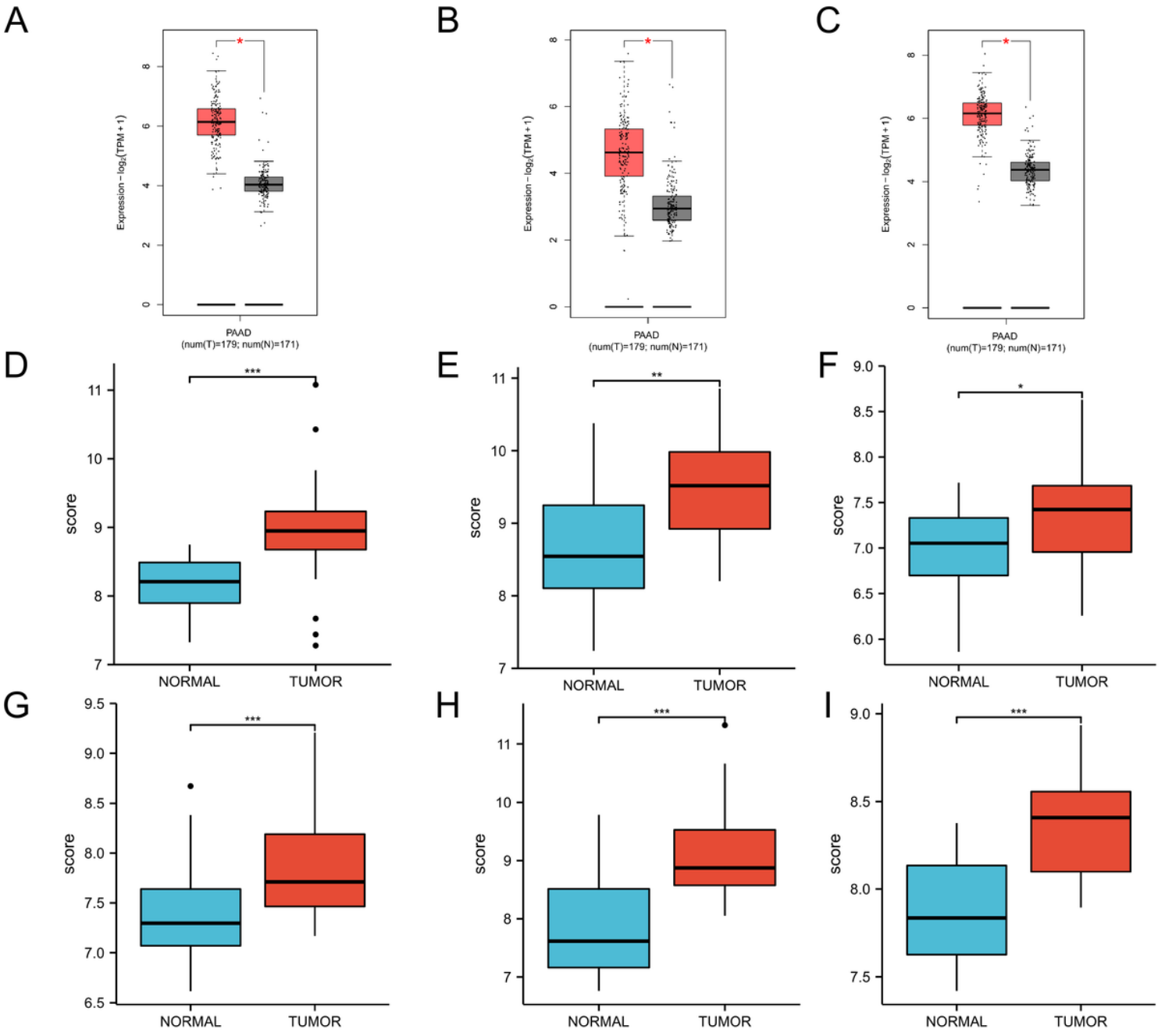

Figure 2

Figure A, figure B and figure C are the differential expression box maps of PLOD1/2/3 pancreatic cancer and normal samples in GEPIA2 database, respectively. Red box, tumor sample; black box, normal sample. T, tumor; N, normal.Figure D, figure E and figure F show the differential expression of PLOD1/2/3 in pancreatic cancer and normal samples in GSE16515 dataset, red box, tumor sample, blue box, normal sample, respectively. Figure G, figure H and figure I show the difference of PLOD1/2/3 expression between pancreatic cancer and normal samples in GSE15471 dataset,red box,tumor sample,blue box, normal sample. (*<0.05,**<0.01,***<0.001).

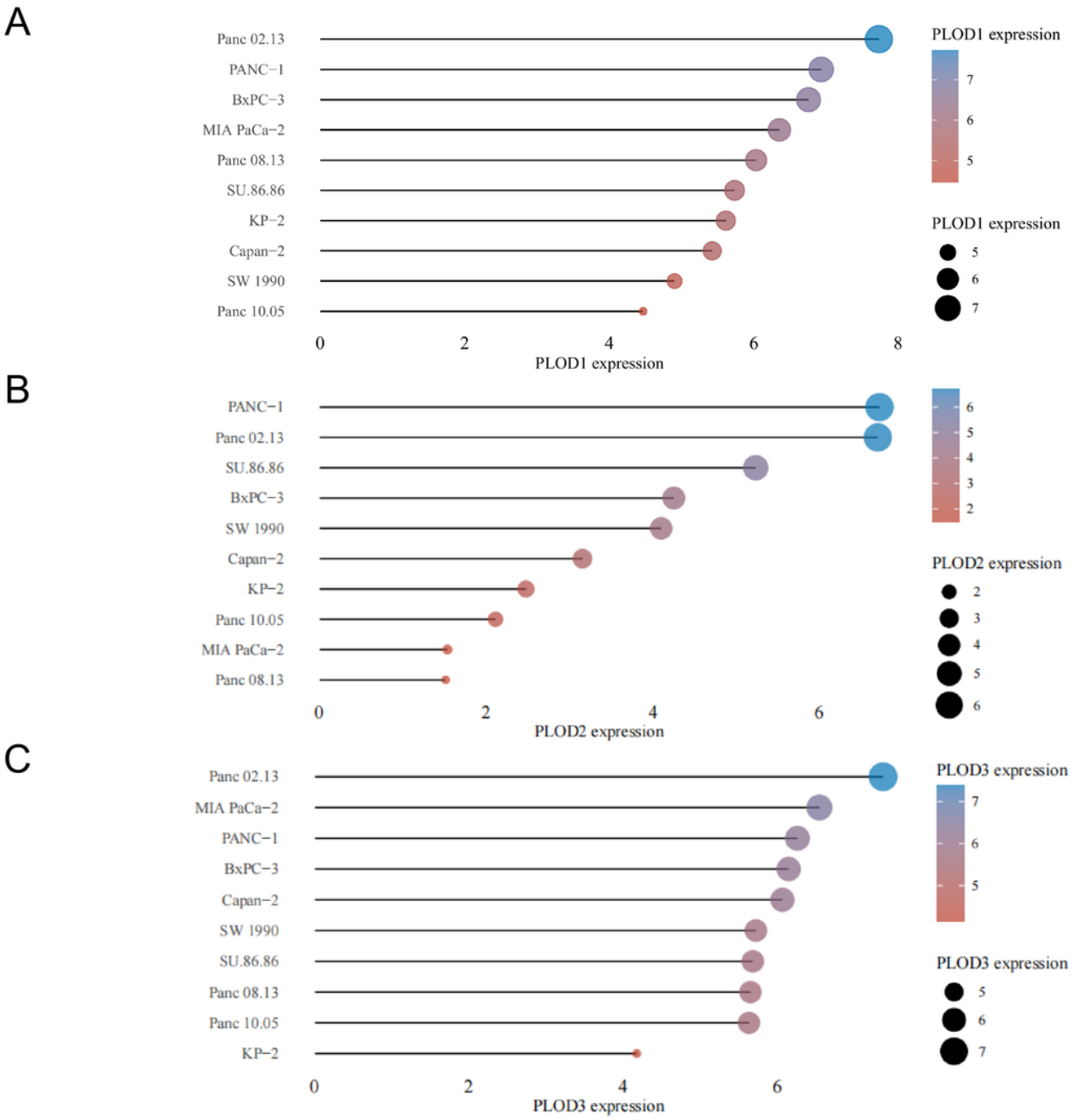

Figure 3

CCLE analyzed the expression of members of the PLODs family in the pancreatic cancer cell lines.Figure A, Figure B and Figure C show the expression of PLOD1/2/3 in pancreatic cancer cell lines, respectively. The horizontal axis in the figure represents the expression of the gene, the ordinate is for different cell lines, the size of the dot in the figure represents the level of expression, and the different colors also represent the level of expression.

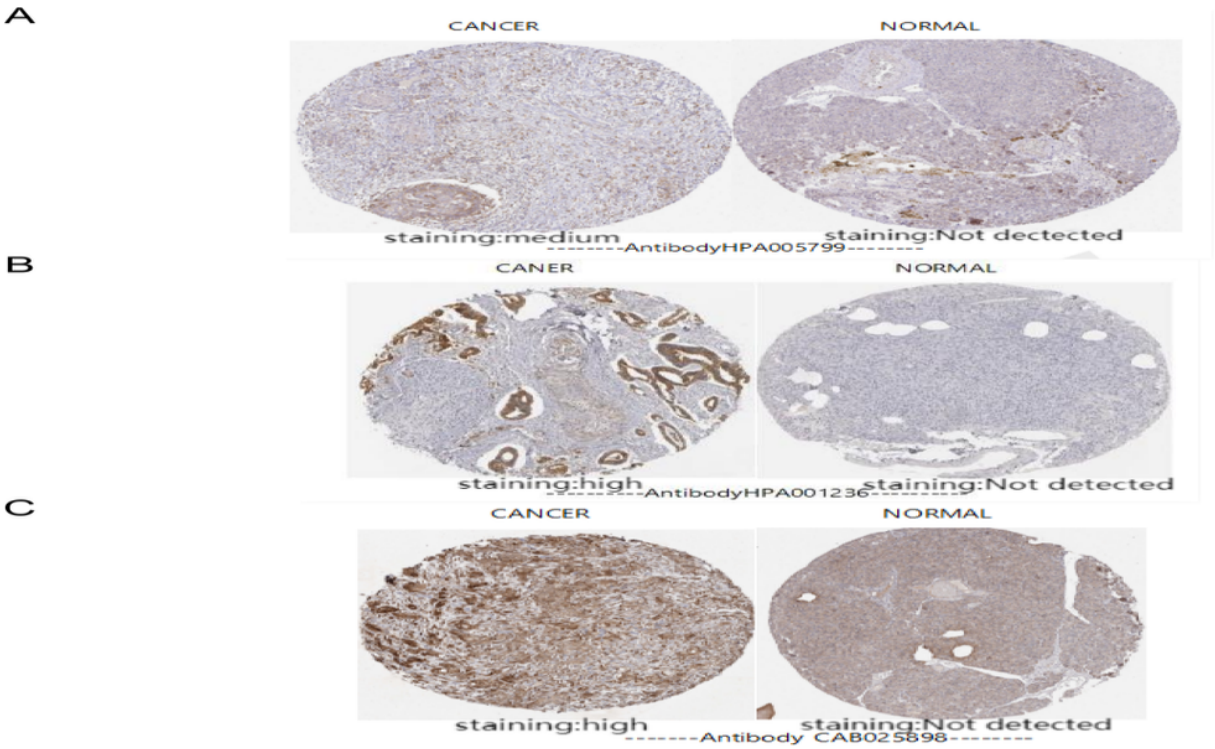

Figure 4

IHC images of different members of the PLOD family were detected using Human Protein Atlas (HPA)Figure A shows PLOD1 in pancreatic cancer and not in normal pancreatic tissue. Figure. B and C are highly expressed by PLOD2 and PLOD3 in pancreatic cancer and are not expressed in normal pancreatic tissue.

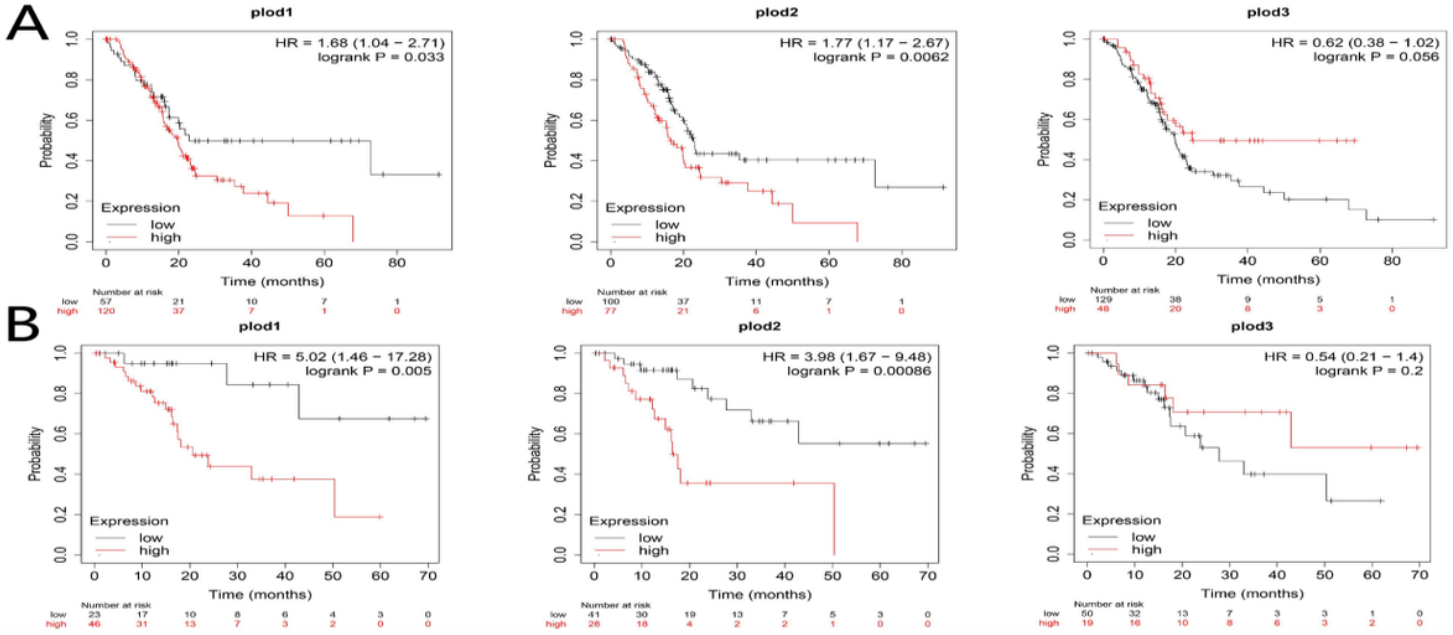

Figure 5

The survival analysis of PLOD family members in PAAD. (A) Overexpression levels of PLOD1 and PLOD2 were associated with shorter OS in PAAD. (B) Overexpression levels of PLOD1 and PLOD2 were associated with shorter RFS in PAAD.

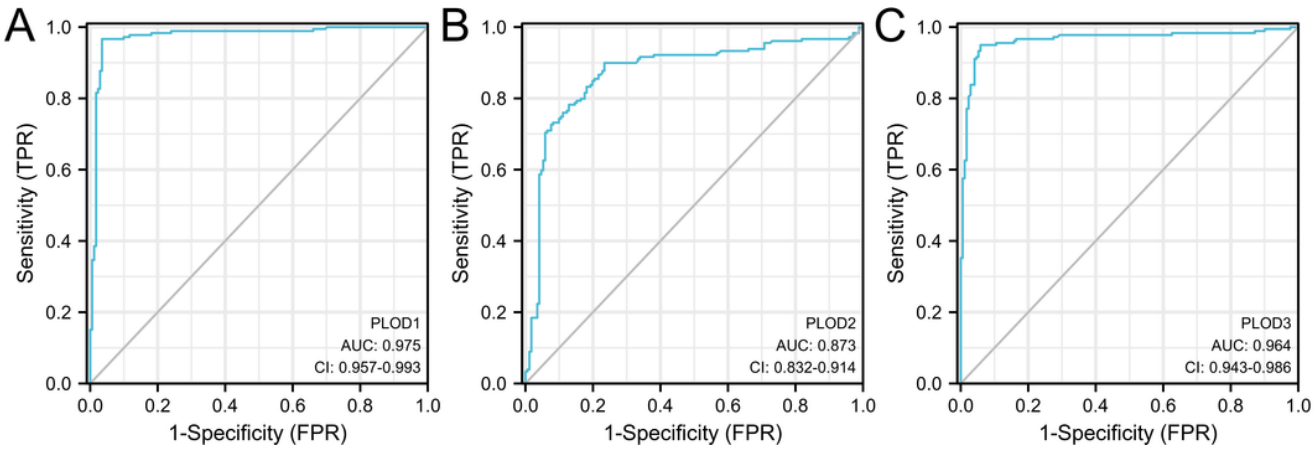

Figure 6

PLODs has high accuracy in predicting the outcome of Normal and Tumor.Figures A, B and C are the ROC curves of PLOD1,PLOD2 and PLOD3 respectively.

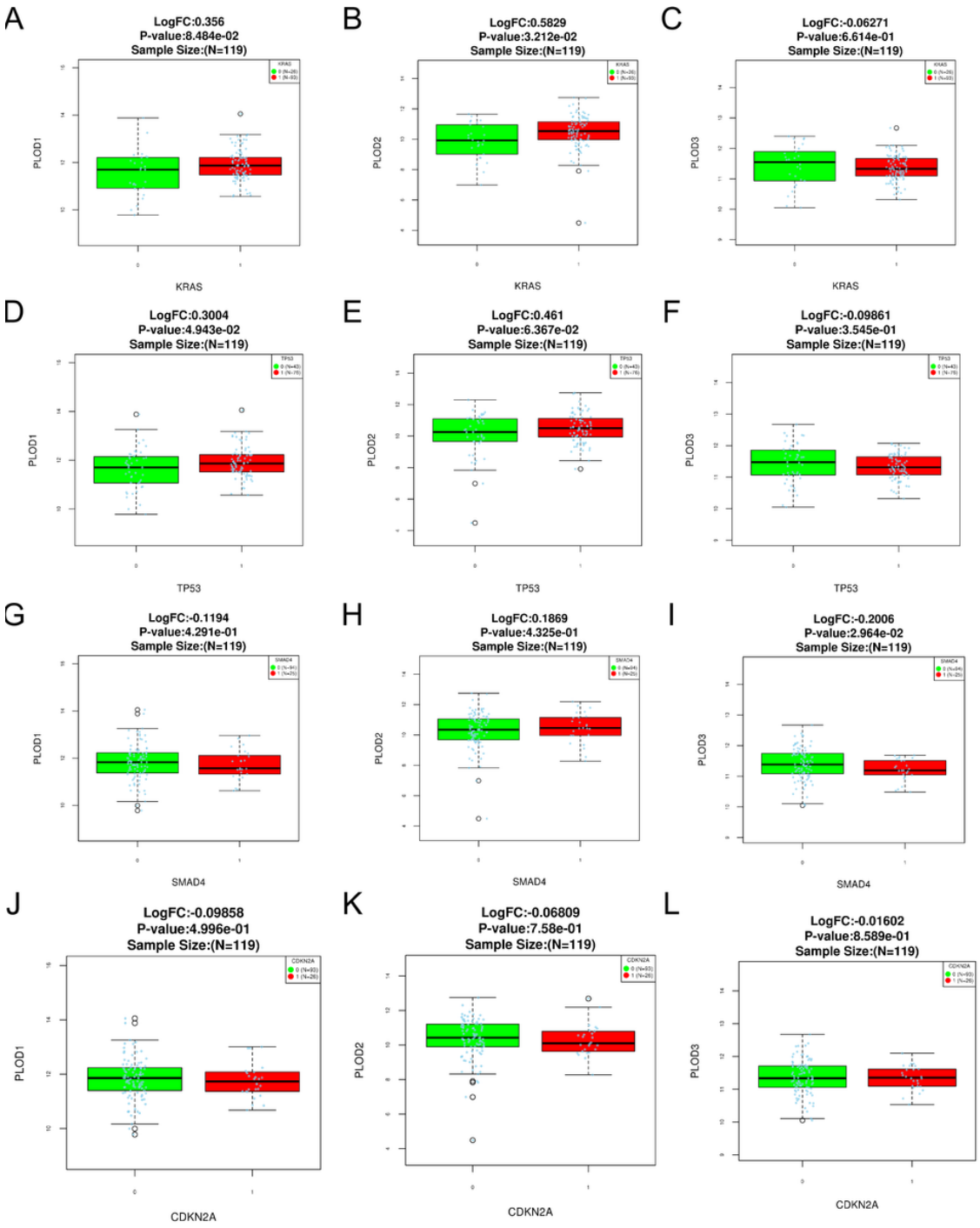

Figure 7

Green BOX represents KRAS,TP53,SMAD4,CDKN2A wild-type pancreatic cancer, and red BOX represents KRAS,TP53,SMAD4,CDKN2A mutant pancreatic cancer. 0 is wild type and 1 is mutant.

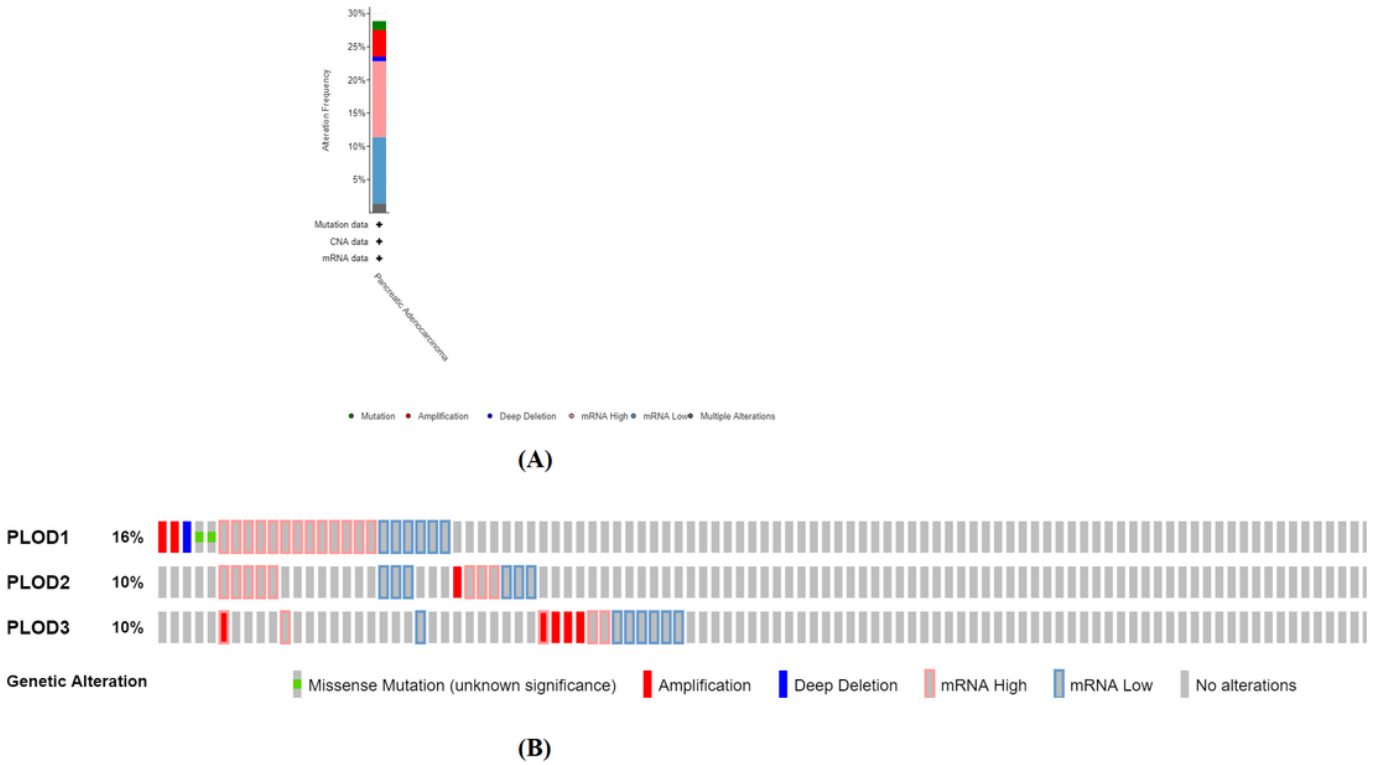

Figure 8

(A)Summary of alterations in PLOD family members in PAAD. (B) mRNA expression alterations (RNA Seq V2 RSEM) of the PLOD family genes in PAAD patients.

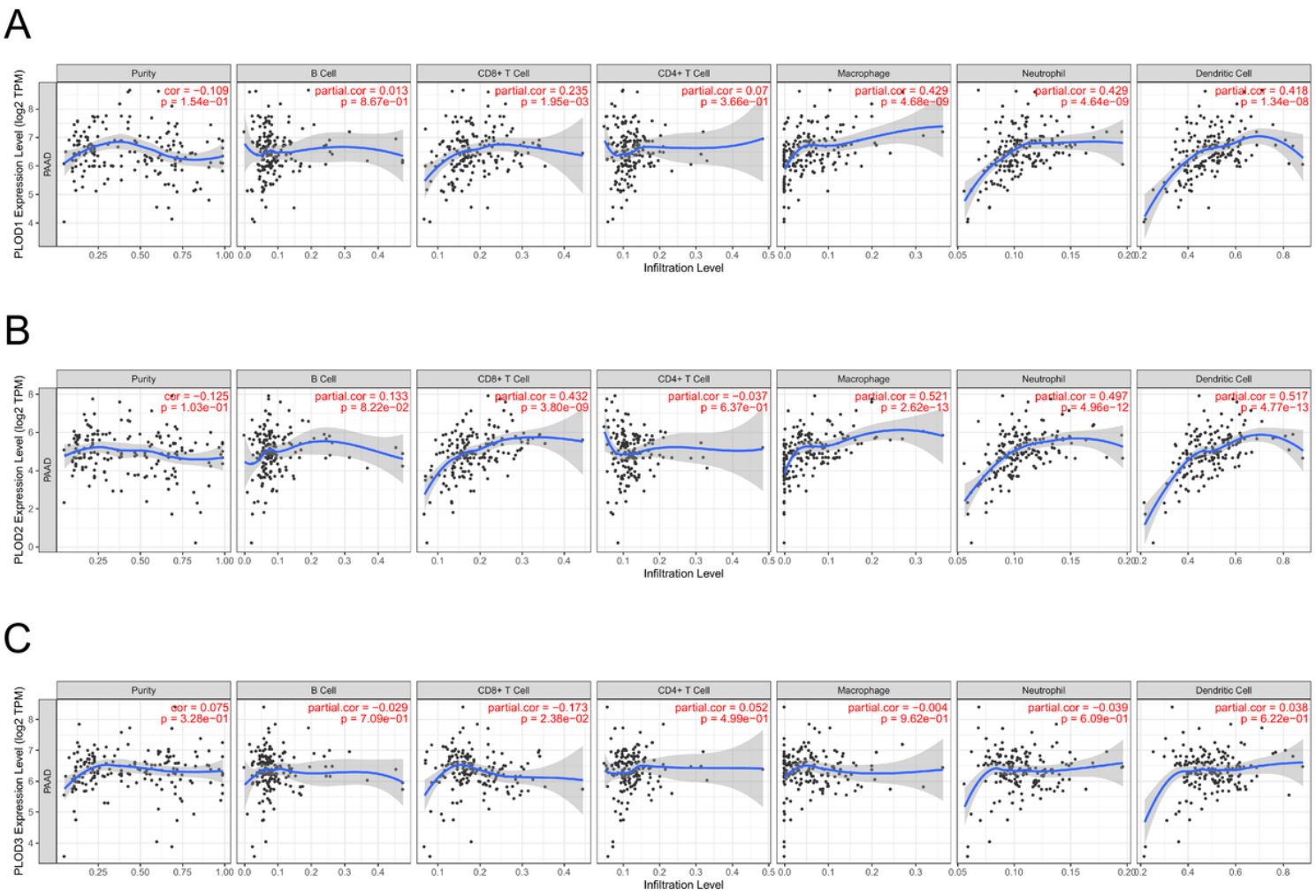

Figure 9

(A)The correlation between each type of TIICs (B-cells, CD4+ T-cells, CD8+ T-cells, neutrophils, macrophages and dendritic cells) and PLOD1. (B) The correlation between each type of TIICs (B-cells, CD4+ T-cells, CD8+ T-cells, neutrophils, macrophages and dendritic cells) and PLOD2. (C) The correlation between each type of TIICs (B-cells, CD4+ T-cells, CD8+ T-cells, neutrophils, macrophages and dendritic cells) and PLOD3.

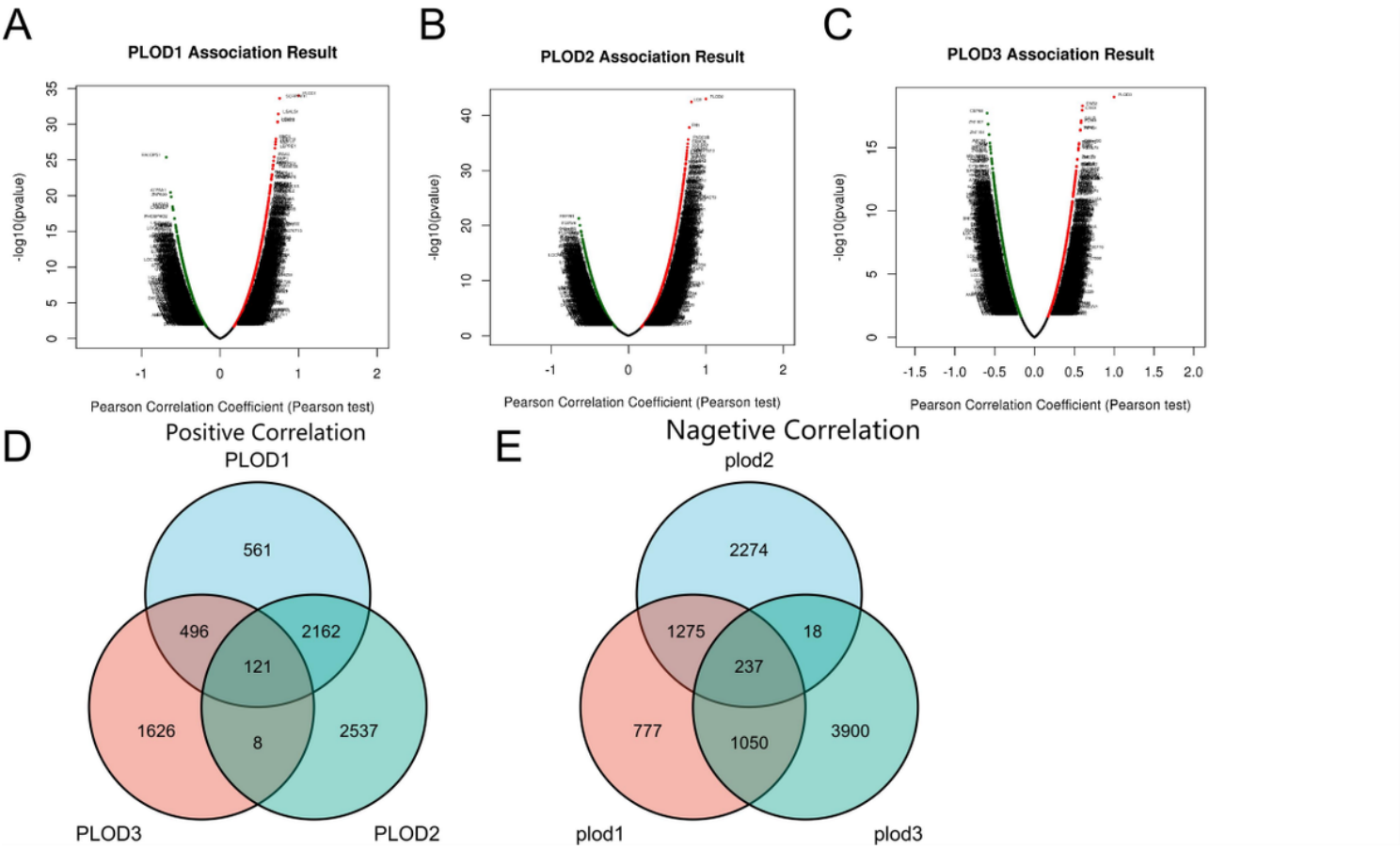

Figure 10

Signal modules associated with the PLOD family gene in the PAAD. The (A), (B), (C) volcanic maps show the coexpressed genes associated with PLOD1, 2 and 3 in the PAAD cohort in the TCGA database. The Pearson correlation test was used to analyze the differences between PLOD genes and differential expression genes in PAAD. Green, powder, and red dots show the genes with the correlation coefficients <-0.25,> 0.25, and <0.5, and> 0.5, respectively. (D) Venn diagram shows positive (left) and (E) Venn diagram negative co-expression genes associated with PLOD1, 2 and 3.

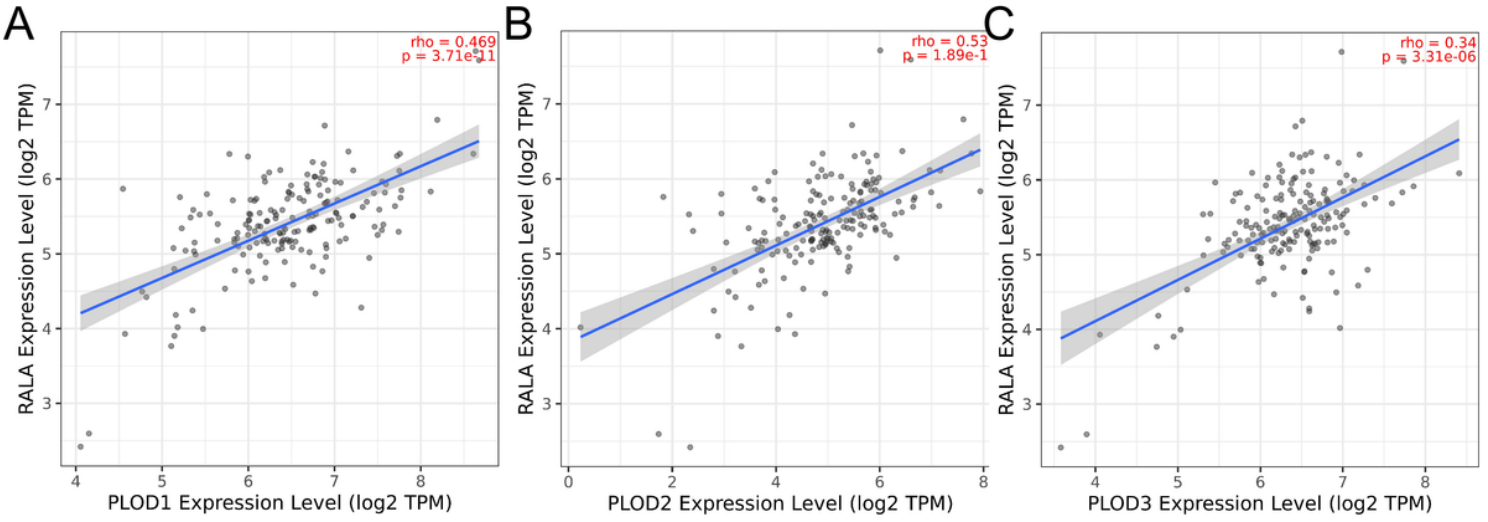

Figure 11

Figure. A PLOD1 and Figure B PLOD2 are associated with RALA moderate, and the Pearson correlation coefficients are rho=0.469 and rho=0.532. Figure C PLOD3 and RALA low degree correlation rho=0.34, respectively

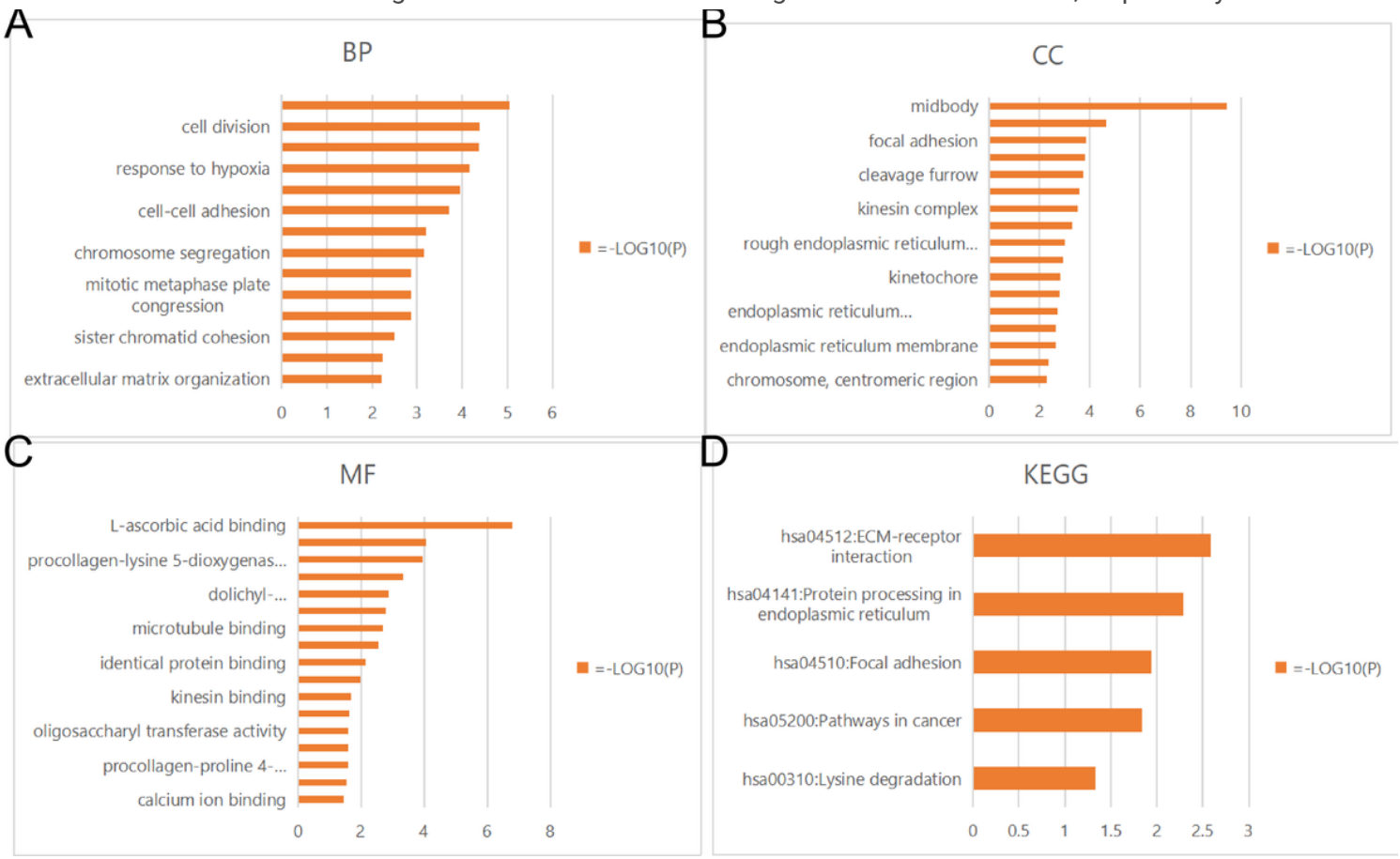

Figure 12

The entries GO (A) BP, (B) CC and (C) MF terms and (D) KEGG pathway. GO, gene ontology; KEGG, Kyoto Encyclopedia of Gene and Genomics; BP, biological processes; CC, cell composition; MF, molecular function.

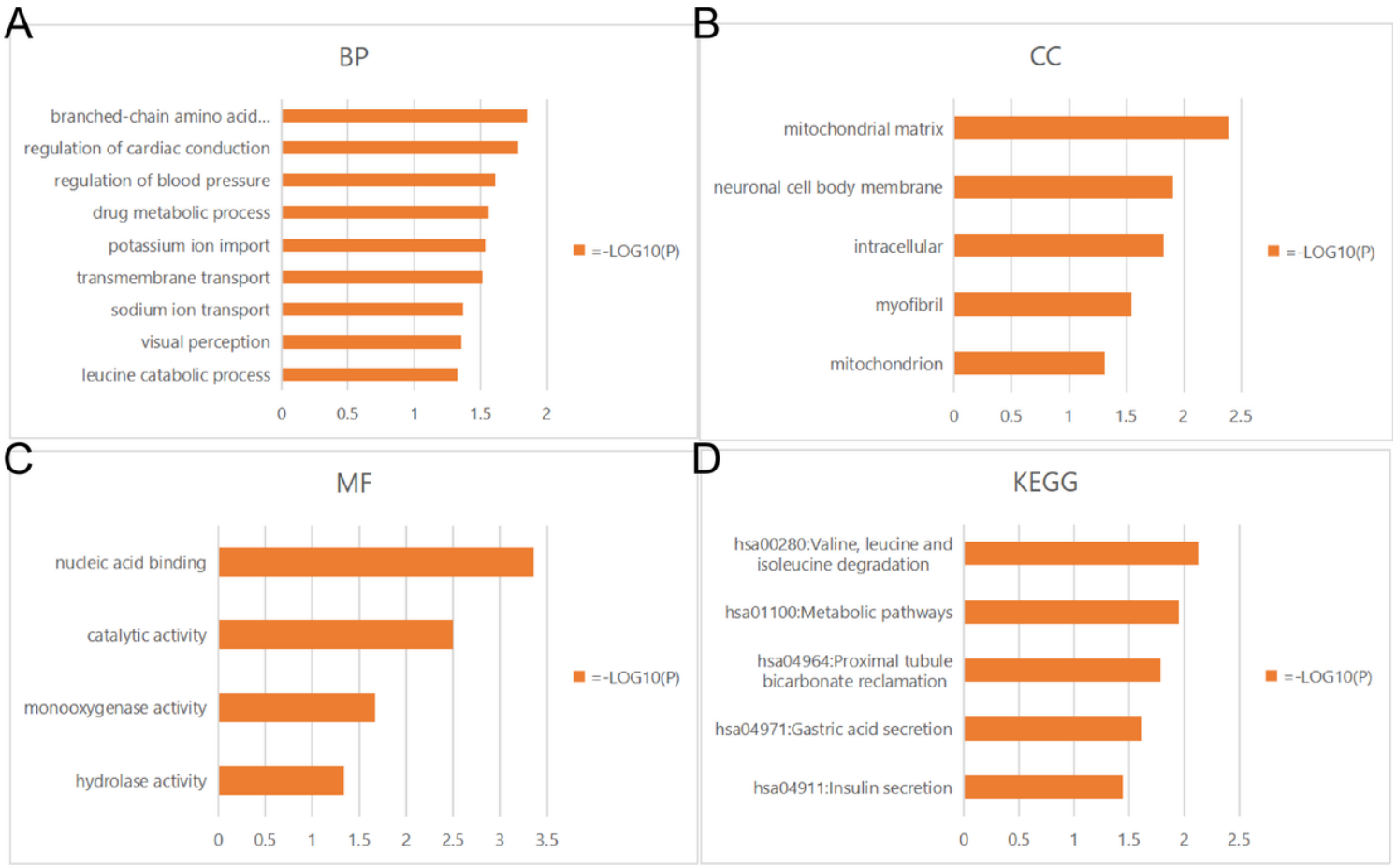

Figure 13

Entry GO (A) BP, (B) CC and (C) MF terms and (D), (D) KEGG pathway. GO, gene ontology; KEGG, Kyoto Encyclopedia of Gene and Genomics; BP, biological processes; CC, cell composition; MF, molecular function.

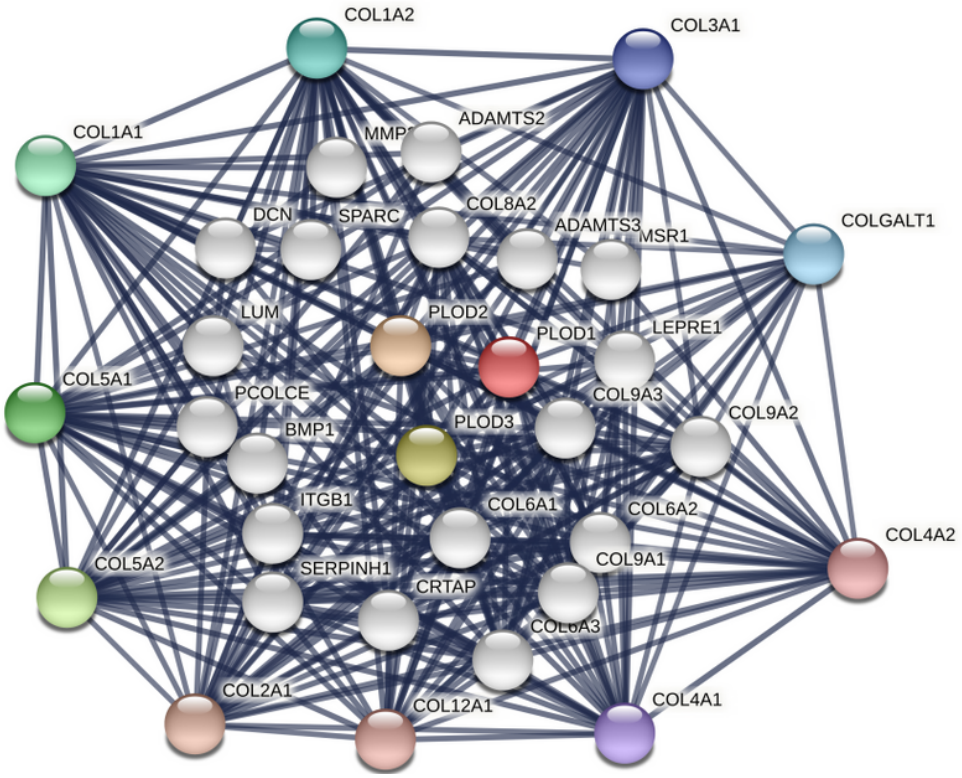

Figure 14

Protein interaction network of 30 functional partners with confidence score > 0.9 based on STRING database. PLOD1, 2 and 3 are the seed genes. Ten interacting partners with the highest confident scores were colored and placed in the outer shell. The other twenty interacting partners were placed in the inner shell. The blue lines represent the correlation between proteins and the thickness of the lines indicates the strength of data support..

Supplementary Files

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

- SupplementaryTable1..docx
- SupplementaryTable2..docx
- SupplementaryTable3.docx
- SupplementaryTable4.docx