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ORIGINAL ARTICLE

Identification of LIPH as an unfavorable biomarkers correlated with immune suppression or evasion in pancreatic cancer based on RNA-seq

Hongkai Zhuang[1,2] · Xinming Chen[3] · Ying Wang[4] · Shanzhou Huang[2] · Bo Chen[5] · Chuanzhao Zhang[2] · Baohua Hou[2]

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Abstract

Background It is widely considered that pancreatic cancer (PC) is an immunosuppressive cancer. Immune-based therapies remain promising therapeutic strategies for PC. Overexpression of lipase H (LIPH) was reported to be related to immunity in cattle and has also been demonstrated to promote tumor progression in several tumors, but its role in pancreatic carcinogenesis remains unclear. Study on LIPH in PC might provide a new insight into the immunosuppression in PC.

Methods The potential biological and clinical significance of LIPH was evaluated by bioinformatics analysis. We further investigated potential associations between the expression of LIPH and tumor immune infiltration using the CIBERSORT algorithm, the ESTIMAT algorithm, and single sample gene set enrichment analysis (ssGSEA).

Results LIPH was significantly overexpressed in tumor tissues compared with normal tissues. LIPH overexpression correlated with tumor recurrence, advanced histologic grade, and poorer overall survival (OS). Four of the most common somatic mutation, including KRAS, TP53, CDKN2A, and SMAD4, in PC were all correlated with high LIPH expression. And high LIPH expression was significantly correlated with KRAS activation and SMAD4 inactivation. Besides, LIPH expression was involved in various biological pathways such as negative regulation of cell–cell adhesion, actin cytoskeleton, EMT, angiogenesis, and signaling by MST1. And LIPH overexpression caused high infiltration of TAMs, Treg cells, and Th2/Th1, but reduced the infiltration of $CD8^+$ T cells and Th1 cells.

Conclusions Our findings demonstrated that LIPH correlated with immune suppression or evasion and may function as a novel unfavorable prognostic biomarker in PC.

Keywords Pancreatic cancer (PC) · Lipase H (LIPH) · The Cancer Genome Atlas (TCGA) · The Gene Expression Omnibus (GEO) · Immune infiltration

Hongkai Zhuang, Xinming Chen and Ying Wang co-first authors.

✉ Bo Chen
chenbo@gdph.org.cn

✉ Chuanzhao Zhang
641703837@qq.com

✉ Baohua Hou
15917919681@163.com

[1] Department of Hepatobiliary Surgery, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510080, China

[2] Department of General Surgery, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, No. 106 Zhongshan Er Road, Guangzhou 510080, China

[3] Department of Hepatobiliary Surgery, Shenshan Central Hospital, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Shanwei 516600, China

[4] The Affiliated Hospital of Stomatology, School of Stomatology, Zhejiang University School of Medicine, Hangzhou 310006, China

[5] Department of Breast Cancer, Cancer Center, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, No. 106 Zhongshan Er Road, Guangzhou 510080, China

Introduction

As one of the most aggressive malignancies, pancreatic cancer (PC) causes nearly 5% of all cancer-related deaths worldwide [1]. Poor survival is attributed to its high aggressiveness and chemotherapeutic resistance [2]. Presently, surgery remains the major therapy for PC, but only 20% of PC patients present with surgically resectable status, 80% among which die within 5 years [3]. PC is a highly immunosuppressive cancer and unique from an immunological perspective [4]. Immune-based therapies that recruit or enhance antitumor immune cells in the tumor microenvironment (TME) remain novel therapeutic strategies for PC [5]. In recent years, immune checkpoint inhibitors (ICIs) have been promising potent drugs in the treatment of several solid tumors, such as malignant melanoma, non-small cell lung cancer, hepatocellular carcinoma, triple negative breast cancer, and head-neck squamous cell carcinoma, but so far, lack of efficacy in advanced PC patients [6–12]. Therefore, a deeper understanding of the molecular mechanisms involved in immune suppression is required, which would helpful to develop immune-based therapies and improve prognosis in PC [13].

LIPH, also known as mPA-PLA1, is a protein coding gene that encodes a membrane-bound protease that can catalyze the production of 2-acyl lysophosphatidic acid (LPA) [14, 15]. Moreover, LPA was reported as a lipid mediator with diverse biological properties that include proliferation, migration, survival, and angiogenesis in multiple cancers [16]. Early studies about LIPH focused on its mutation and its correlation with hypotrichosis [17, 18]. Besides, Orozco-terWengel et al. reported that LIPH was related to immunity in cattle [19]. However, oncologic researches about LIPH have been conducted in only four cancers (e.g., breast cancer, lung cancer, papillary thyroid carcinoma, and esophageal adenocarcinoma) [14, 20–22]. The precise biological mechanism of LIPH in PC progression remains poorly understood.

In the present study, we systemically analyzed the expression pattern of LIPH and potential biological role in PC. For the first time, potential correlation between LIPH expression and immune cells infiltration levels in PC was investigated using the CIBERSORT algorithm, the ESTIMATE algorithm, and ssGSEA [23, 24].

Materials and methods

Data acquisition

The mRNA-sequencing data and clinical information of patients with PC were obtained from the Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/) database [13, 25]. Mutation data on KRAS, TP53, CDKN2A, and SMAD4 in TCGA PC dataset were obtained from cBioportal database (http://www.cbioportal.org/) [25]. Of the 177 PC cases in TCGA PC dataset, 171 were patients with OS > 1 month [25]. In addition, several GEO datasets, including GSE79668, GSE28735, GSE60979, and GSE62452, were selected for further analysis. All datasets are freely available as public resources. Consequently, local ethics approval was not required.

LIPH expression analysis

Differential expression analysis for LIPH was, respectively, performed in GSE60979 and GSE62452 datasets. Then, LIPH expression in PC was further validated in the Oncomine database (https://www.oncomine.org/resource/main.html) and Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancerpku.cn/index.html). And GSE28735 dataset (45 pairs of adjacent non-tumor tissues and pancreatic tumor) was used for paired differential expression analysis of LIPH. Moreover, the Human Protein Atlas database (http://proteinatlas.org/) was used to further validate the protein expression of LIPH and the expression of LIPH in cancer cell lines.

Survival analysis

Kaplan–Meier (KM) survival analysis was conducted to investigate the correlation between LIPH expression and OS of PC patients in TCGA PC dataset, GSE79668 dataset, and GSE62452 dataset. The optimal cutoff points were, respectively, obtained from the X-tile 3.6.1 software (Yale University, New Haven, CT, USA), and patients were, respectively, divided into low expression (low-Exp) and high expression (high-Exp) groups [13, 26].

Association between LIPH expression and somatic mutation

Early studies reviewed that KRAS, TP53, CDKN2A, and SMAD4 mutation are four of the most frequent genetic alterations for PC [25, 27]. Firstly, we evaluated the association between these mutation statuses and LIPH expression. Then, we also evaluated the association between LIPH expression

and these four genes in TCGA PC dataset and GSE62452 dataset. Besides, we also assessed the association between the total mutational burden (TMB) and LIPH expression. In summary, we tried to preliminarily figure out whether somatic mutation level had an influence on the expression of LIPH in PC.

Functional enrichment analysis of LIPH expression

Co-expression genes of LIPH in TCGA PC dataset and the Broad Institute Cancer Cell Line Encyclopedia (CCLE) were separately screened out with the threshold of |Pearson correlated coefficient|> 0.6 and $P < 0.05$ [13]. Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to figure out the overlapped co-expression genes between TCGA PC dataset and CCLE database. Then, the overlapped co-expression genes were imported in ConsensusPathDB (http://cpdb.molgen.mpg.de/) for functional enrichment analysis; $P < 0.05$ was considered statistically significant [13, 28].

Immune infiltration analysis for TCGA PC dataset

The CIBERSORT algorithm is an analysis tool that assesses specific immune cell fractions using gene expression data [23]. Therefore, the CIBERSORT algorithm was performed to evaluate the infiltration level of 22 immune cell types in TCGA PC dataset. The PC samples with a CIBERSORT output of $P < 0.05$ were included for further study. Subsequently, using R package estimate, the ESTIMATE algorithm was performed to generate an immune score and a tumor purity score [24]. A higher tumor purity score indicates a low level of immune cell infiltration in tumor tissue. PC samples with higher immune scores showed higher infiltration level of immune cells in tumor tissues [25]. Then, using R package gsva, ssGSEA was performed to assess the enrichment levels of immune-related terms in the cancer samples [29]. The following 29 immune-related terms were obtained: tumor-infiltrating lymphocyte (TIL), $CD8^+$ T cells, regulatory T cells (Treg), cytolytic activity, type-2 T helper cells (Th2 cells), T cell co-stimulation, type-1 T helper cells (Th1 cells), T cell co-inhibition, checkpoint, natural killer cells (NK cells), tumor-associated macrophages (TAMs), antigen-presenting cell (APC) co-stimulation, major histocompatibility complex (MHC) class-1, antigen-presenting cell (APC) co-inhibition, follicular helper T cells (Tfh), type-1 IFN response, dendritic cells, parainflammation, plasmacytoid dendritic cells (pDCs), activated dendritic cells (aDCs), immature dendritic cells (iDCs), mast cells, B cells, neutrophils, inflammation-promoting, human leukocyte antigen (HLA), T helper cells, type-2 IFN response, and chemokine receptor (CCR) [30]. Using R package sparcl, TCGA PC dataset was divided into three clusters—immunity L, immunity M, and immunity H—according to the enrichment scores of 29 immune-related terms. Moreover, GSE62452 dataset was utilized to validate the immune infiltration of PC through the ESTIMATE algorithm and ssGSEA method.

Statistical analysis

All statistical analyses were performed using R software (http:///www.r-project.org/), GraphPad prism 8.0 software (San Diego, CA, USA), and SPSS 25.0 software (Chicago, IL, USA). The chi-square test or Fisher's exact test and contingency analysis were used to assess the association between LIPH expression and clinicopathological features. Correlations were assessed using Pearson correlated coefficient. Group differences were evaluated by the Student's *t*-test and expressed as mean ± SD. Statistical significance was defined by a value of $P < 0.05$.

Results

LIPH overexpression predicts poor prognosis in PC

In GSE62452 and GSE60979 datasets, LIPH was overexpressed in PC tissues compared with normal pancreatic tissues ($P < 0.0001$) (Fig. 1A, B). Three studies in the Oncomine database showed that LIPH was significantly upregulated in PC tissues ($P < 0.05$) (Fig. 1C–E). And similar result was found in the GEPIA database (Fig. 1F). In GSE28735 dataset, LIPH was overexpressed in tumor tissues when compared with that in the adjacent non-tumor tissues ($P < 0.0001$) (Fig. 1G). We also used the Human Protein Atlas database to investigate the protein expression of LIPH and the expression of LIPH in cancer cell lines, and demonstrated that the protein expression of LIPH in PC tissues was significantly upregulated (Fig. 2A, B), and CAPAN-2 cells showed much higher LIPH expression compared with various other cancer cells (Fig. 2C). Of note, patients with lower expression of LIPH had a better survival than those with higher expression of LIPH ($P < 0.05$) (Fig. 3A–C). Taken together, the current study identified LIPH as an unfavorable prognostic factor for patient with PC.

Correlation between LIPH expression and the clinicopathological characteristics of PC

The correlation between LIPH expression and clinicopathological characteristics of PC is shown in Table 1. Higher expression of LIPH was significantly correlated with advanced histologic grade ($P = 0.000008$) and tumor recurrence ($P = 0.001$). These results indicated that LIPH overexpression was associated with tumor progression in PC.

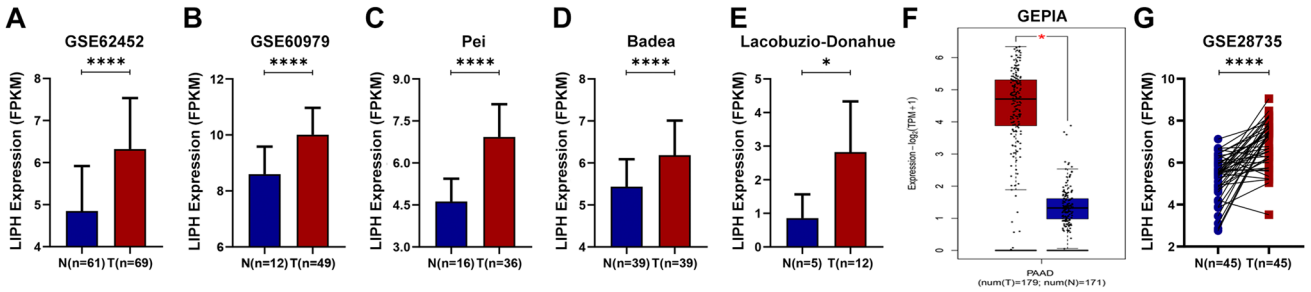

Fig. 1 Multiple databases demonstrated that LIPH was overexpressed in PC. **A**, **B** The expression of LIPH in PC was significantly upregulated in PC tissues compared with that in normal tissues in both the GSE62452 and the GSE60979 datasets. **C**–**E** Three studies in the Oncomine database demonstrated that LIPH was overexpression in PC tissues. **F** GEPIA database demonstrated that the expression of LIPH was significantly higher in PC tissues than that in normal tissues. **G** GSE28735 dataset demonstrated the expression LIPH was upregulated in PC tissues compared with that in the adjacent non-tumor tissues. (*$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$). PC, pancreatic cancer; LIPH, lipase H; N, normal; T, tumor

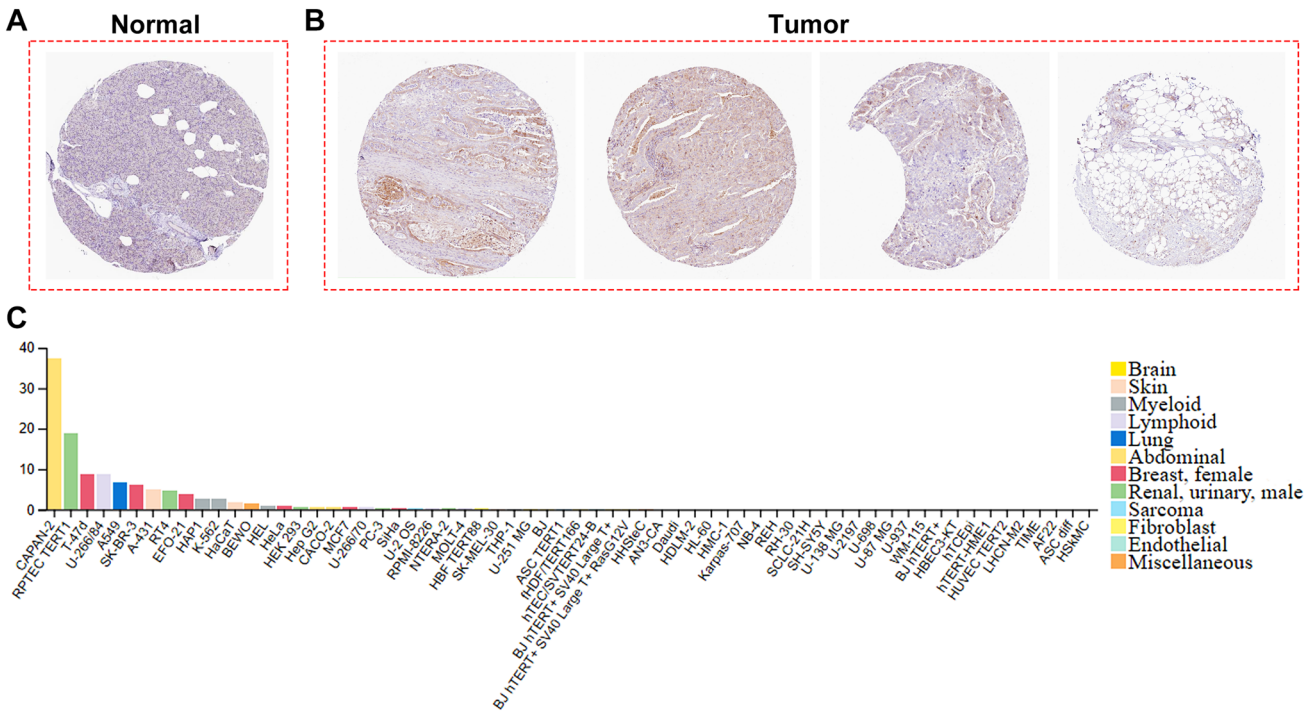

Fig. 2 The Human Protein Atlas database analysis. **A**, **B** The Human Protein Atlas database was used to validate the protein expression of LIPH, which demonstrated that the protein expression of LIPH was significantly upregulated in tumor tissues. **C** The expression of LIPH in CAPAN-2 cells was much higher than that in various other cancer cells

Association between LIPH expression and somatic mutation

The mutation landscape of PC in TCGA PC dataset is shown in Figure S2, and consistently, KRAS, TP53, CDKN2A, and SMAD4 were four of the most frequent genetic alterations for PC. Our study showed that KRAS, TP53, CDKN2A, and SMAD4 mutation status were significantly correlated with LIPH overexpression (Fig. 4A). Differential expression analysis for these 4 genes between the high- and low-Exp LIPH groups in TCGA PC dataset revealed that KRAS was significantly upregulated in high-Exp group, while SMAD4 was significantly downregulated in high-Exp group (Fig. 4B). And correlation analyses demonstrated positive correlation between LIPH expression and KRAS expression ($Cor=0.5$, $P<0.05$), while negative correlation between LIPH expression and SMAD4 expression ($Cor=-0.44$, $P<0.05$) (Fig. 4C). Similar results were found in GSE62452

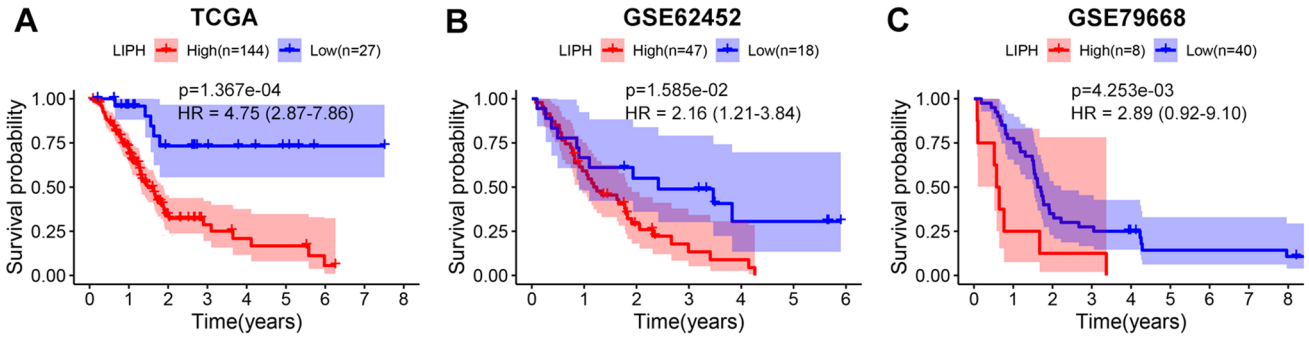

Fig. 3 KM survival analysis for LIPH through TCGA, GSE62425, and GSE79668 datasets. LIPH overexpression was significantly associated with poor survival in PC (P < 0.05). KM, Kaplan–Meier; HR, hazard ratio

dataset (Figure D, E). Besides, the correlations among LIPH, KRAS, and SMAD4 were also validated in cBioportal database, which were consistent with our findings (Figure S3A-B). In addition, Figure S4 demonstrated that LIPH expression was significantly correlated with TMB. These results suggested that LIPH highly correlated with KRAS and SMAD4, and thereby promoted tumor progression through cooperating with KRAS and SMAD4.

Functional enrichment analysis of LIPH expression in PC

We further investigated the biological role of LIPH in PC. We conducted co-expression analysis (Pearson correlated coefficient|> 0.6, $P < 0.05$) for LIPH based on TCGA PC dataset and the CCLE database. Significantly overlapped co-expression genes (33 overlapped co-expression genes; see in Fig. 5A, Table 2) were imported in ConsensusPathDB and subjected to functional enrichment analysis ($P < 0.05$) (Fig. 5B). GO enrichment analysis showed that LIPH might play a vital role in negative regulation of cell–cell adhesion, regulation of Notch signaling pathway, actin cytoskeleton, lipid phosphorylation, and glycerophospholipid metabolic process (Fig. 5B). Moreover, pathway enrichment analysis revealed that LIPH may be important for the regulation of cell adhesion molecules, epithelial to mesenchymal transition (EMT), epidermal growth factor receptor 1 (EGFR1), and signaling by macrophage stimulating 1 (MST1) (Fig. 5B). These results implied that the expression of LIPH provided necessary support for tumorigenesis, progression, and immune infiltration in PC.

LIPH overexpression correlates with immune suppression or invasion in PC

Through CIBERSORT algorithm, 127 PC samples with a CIBERSORT output P < 0.05 were involved in the assessment of 22 immune cells (ICs) types infiltration. And we found that low infiltration level of CD8 + T cells or high infiltration of TAMs significantly correlated with poor prognosis in PC ($P < 0.05$), consistent with previous studies (Fig. 6A, B). And our study showed that LIPH overexpression associated with lower infiltration level of $CD8^+$ T cells (Cor = −0.27, $P < 0.01$), but higher infiltration level of TAMs (Cor = 0.18, $P < 0.05$) (Fig. 6C, D). ESTIMATE algorithm showed that LIPH overexpression correlated with higher tumor purity (Cor = 0.27, $P < 0.001$), but lower immune score (Cor = −0.20, $P < 0.01$) (Fig. 6E, F), a finding which was also found in GSE62452 dataset (Fig. 7A, B). Using ssGSEA method, the enrichment level of 29 immune-related terms was obtained and 177 PC samples in TCGA PC dataset were divided into immunity L ($n = 115$), immunity M ($n = 55$), and immunity H ($n = 7$) (Fig. 6G). We merged immunity M and immunity H into immunity M/H ($n = 62$) and found that the expression of LIPH in immunity M/H was much lower than that in immunity L ($P < 0.05$) (Fig. 6H). In TCGA PC dataset, ssGSEA analysis demonstrated that LIPH overexpression was significantly associated with low infiltration levels of $CD8^+$ T cells and Th1 cells (Fig. 6I, J). In contrast, high infiltration levels of TAMs, Treg cells, and Th2/Th1 were significantly associated with high LIPH expression ($P < 0.05$) (Fig. 6K, M). Similar results were observed in the GSE62452 dataset (Fig. 7C–E). These results suggested that LIPH overexpression promoted immune suppression or invasion in PC.

Discussion

Pancreatic cancer (PC) is one of the most aggressive cancers with a very poor prognosis, and its incidence is rising every year [31]. It is consistently considered that PC is a "cold" tumor, which is highly immunosuppressive [32]. Understanding of the molecular mechanisms involved in PC immune suppression is believed to be helpful to develop novel immune-based therapies for PC [33]. The present

Table 1 Correlation of LIPH expression to clinicopathological features in PC

Parameters	LIPH expression		*P*
	Low ($n=27$)	High ($n=144$)	
Age			
≦60	9 (33.3%)	48 (33.3%)	1.00
> 60	18 (66.7%)	96 (66.7%)	
Gender			
Female	13 (48.1%)	65 (45.1%)	0.77
Male	14 (51.9%)	79 (54.9%)	
AJCC stage			
I–IIa	9 (33.3%)	38 (26.4%)	0.33
IIb–IV	16 (59.3%)	105 (72.9%)	
Unknown	2 (7.4%)	1 (0.7%)	
Histologic grade			
G1	11 (40.7%)	17 (11.8%)	0.000008****
G2	10 (37.0%)	82 (56.9%)	
G3	3 (11.1%)	44 (30.6%)	
G4	2 (7.4%)	0	
Unknown	1 (3.7%)	1 (0.7%)	
Recurrence			
No	18 (66.7%)	48 (33.3%)	0.001**
Yes	9 (33.3%)	96 (66.7%)	
Alcohol history			
No	10 (37.0%)	52 (36.1%)	0.99
Yes	15 (55.6%)	82 (56.9%)	
Unknown	2 (7.4%)	10 (6.9%)	
Diabetes history			
No	16 (59.3%)	89 (61.8%)	0.77
Yes	5 (18.5%)	31 (21.5%)	
Unknown	6 (22.2%)	24 (16.7%)	
Tumor size			
< 4	17 (63.0%)	73 (50.7%)	0.21
≧4	10 (37.0%)	58 (40.3%)	
Unknown	0	13 (9.0%)	
Tumor site			
Head	17 (63.0%)	116 (80.6%)	0.07
Body and tail	21 (77.8%)	6 (4.2%)	
Unknown	7 (25.9%)	4 2.8%)	

Statistical significance was calculated by the chi-square test and Fisher's extract test

study mainly investigated the role of LIPH during pancreatic carcinogenesis and immunosuppression. Consistent with previous studies about LIPH in other carcinomas [14, 20–22], multiple databases showed that LIPH was significantly upregulated in PC (Figs. 1, 2) and correlated with advanced histologic grade, tumor recurrence, and poor survival of patients with PC (Table 1, Fig. 3). Thus, LIPH could be a critical unfavorable prognostic factor for patients with PC.

Function enrichment analysis showed that LIPH overexpression in PC might take part in various biological processes such as negative regulation of cell–cell adhesion, actin cytoskeleton, EMT, angiogenesis, and signaling by MST1 (Fig. 5B). These findings suggested that LIPH overexpression in PC may promote tumor progression through affecting actin cytoskeleton, cell–cell adhesion, EMT, and angiogenesis. But further experimental studies should be conducted to elucidate the potential mechanisms of LIPH in pancreatic carcinogenesis.

Our study found that LIPH expression correlated with the immune infiltration level in PC. Interestingly, in 2015, Orozco-terWengel et al. reported that LIPH was related to immunity in cattle [19]. But we are the first to demonstrate the correlation between LIPH expression and immune infiltration within tumor. Higher LIPH expression was associated with higher tumor purity score and lower immune score (Fig. 6E, F). The expression of LIPH in immunity L was much higher than that in immunity M/H ($P<0.05$) (Fig. 6H). In PC, there was a strong negative association between LIPH expression and the infiltration of $CD8^+$ T cells, Th1 cells. In contrast, LIPH overexpression upregulated the infiltration level of TAMs, Treg cells, and Th2/Th1 in PC. TAMs and Tregs could induce an immunosuppressive tumor microenvironment (TME) through production of immune suppressive cytokines like transforming growth factor β (TGF-β), interleukin-10 (IL-10), and IL-35 [34–36]. These factors could antagonize the antitumor effects of $CD8^+$ T cells and Th1 cells [37, 38]. These results suggested that LIPH played an important role in the formation of immunosuppressive TME in PC through upregulating the pro-tumor effects of TAMS and Treg cells, and downregulating the antitumor effect of $CD8^+$ T cells and Th1, and therefore influenced PC prognosis.

Our study demonstrated that high infiltration of $CD8^+$ T cells significantly prolonged survival for patients with PC (Fig. 6A, B). Fukunaga et al. also reported that increasing infiltration of $CD8^+$ T cells was significantly correlated with prolonged survival in PC [39]. However, the infiltration of $CD8^+$ T cells is usually rare in the TME of PC. A high number of tumor-associated immunosuppressive cells (e.g., TAMs) functions as a barrier to $CD8^+$ T cells infiltration [40]. Moreover, our data demonstrated that the proportion of $CD8^+$ T cells within tumors was much less than that of TAMs. In our study, high infiltration of TAMs within tumors was observed to correlate with poor prognosis in PC. Cui et al. reported that TAMs played large role in promoting tumor growth and inducing an immunosuppressive microenvironment [34]. Taken together, we suggested that

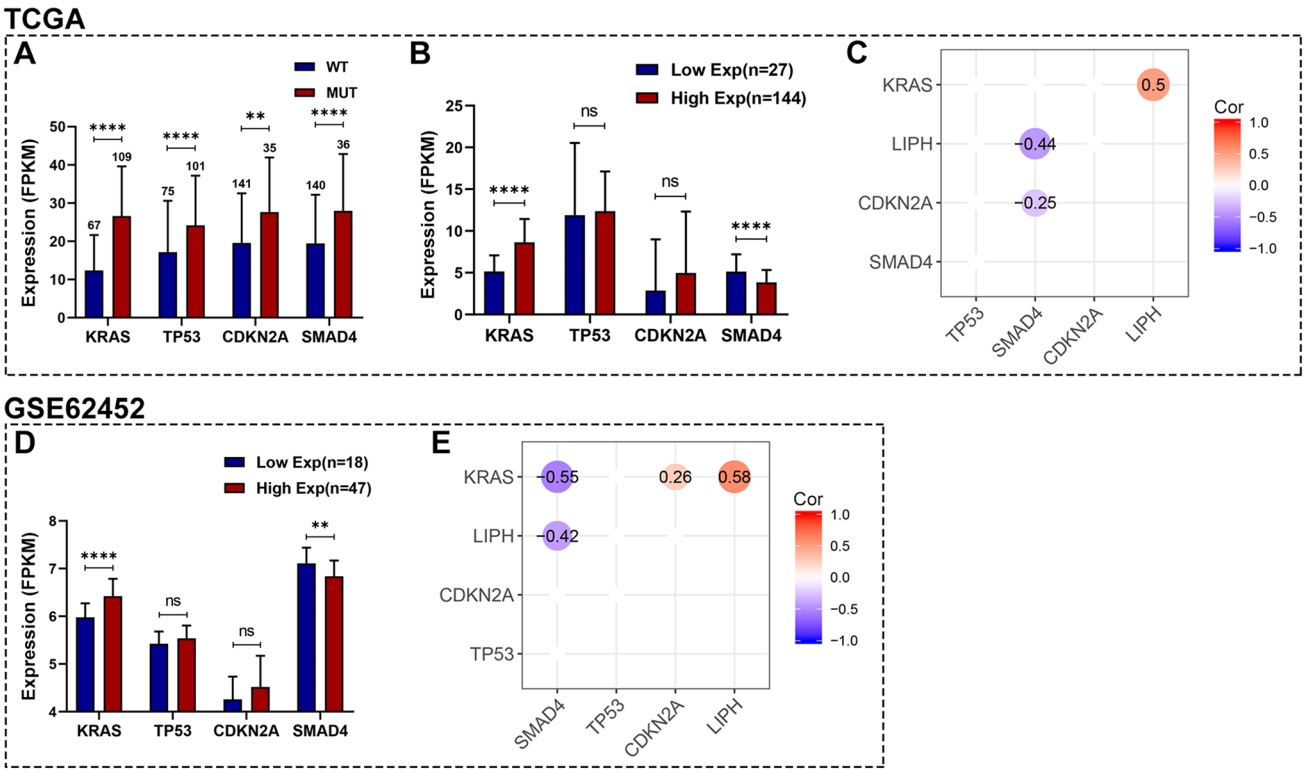

Fig. 4 Association between LIPH expression and somatic mutations. **A** KRAS, TP53, CDKN2A, and SMAD4 mutation status were significantly associated with higher expression of LIPH. **B** Differential expression analysis of KRAS, TP53, CDKN2A, and SMAD4 in high- and low-Exp groups in TCGA PC dataset. **C** Correlation matrix of KRAS, TP53, SMAD4, and CDKN2A in TCGA PC dataset. **D** Differential expression analysis of KRAS, TP53, CDKN2A, and SMAD4 in high- and low-Exp groups in the GSE62452 dataset. **E** Correlation matrix of KRAS, TP53, SMAD4, and CDKN2A in the GSE62452 dataset. (*$P<0.05$; **$P<0.01$;***$P<0.001$; ****$P<0.0001$). PC, pancreatic cancer; TCGA, the Cancer Genome Atlas; Exp, expression; Cor, Pearson correlated coefficient

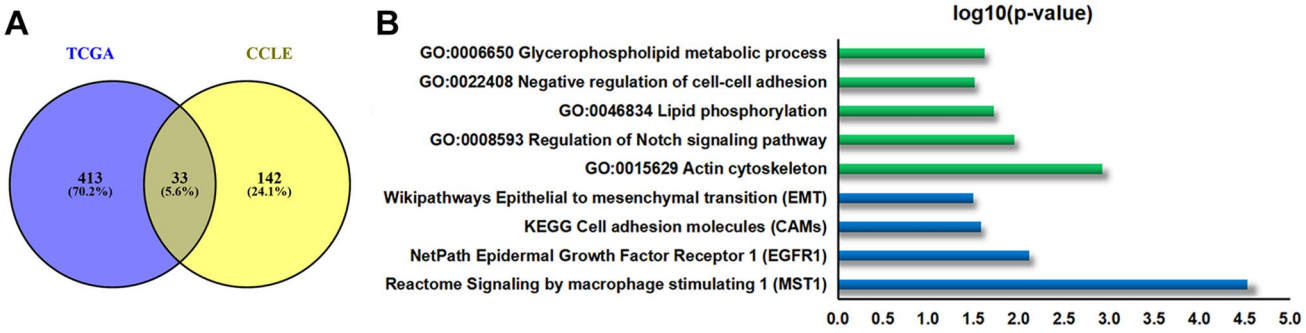

Fig. 5 Co-expression analysis and functional enrichment analysis for LIPH. **A** Venn diagrams showing the co-expression genes of LIPH in TCGA dataset and CCLE database. **B** GO and pathway enrichment analysis for LIPH. TCGA, the Cancer Genome Atlas; CCLE, the Broad Institute Cancer Cell Line Encyclopedia; GO, gene oncology

the antitumor effect of $CD8^+$ T cells in PC was impaired or overwhelmed by the pro-tumor effect of TAMs. Considering TAMs occupying the major proportion of infiltrating ICs within tumors, targeting TAMs could be a promising therapeutic strategy to complement current chemotherapeutic, anti-angiogenic, or ICI therapies.

Through ssGSEA analysis, the significant positive associations between LIPH expression and the infiltration of

Table 2 Co-expression analysis for LIPH (33 overlapped co-expression genes between TCGA dataset and CCLE database)

Gene	TCGA		CCLE	
	Cor	P	Cor	*P*
TIMM22	−0.605	4.56E−19	−0.617	1.73E−05
ABCC3	0.699	2.56E−27	0.65	4.17E−06
AGR2	0.668	2.93E−24	0.614	1.96E−05
ARL14	0.705	6.94E−28	0.698	3.87E−07
B3GNT3	0.703	1.07E−27	0.624	1.29E−05
BCL2L15	0.775	1.19E−36	0.635	8.27E−06
C1orf106	0.82	2.65E−44	0.67	1.66E−06
CDH1	0.663	9.39E−24	0.641	6.42E−06
CGN	0.774	1.47E−36	0.644	5.61E−06
CLDN4	0.68	2.10E−25	0.693	5.05E−07
ELF3	0.722	8.13E−30	0.725	8.50E−08
EPS8L3	0.719	1.88E−29	0.649	4.40E−06
ERBB3	0.736	1.75E−31	0.742	2.82E−08
GPR35	0.7	2.15E−27	0.645	5.34E−06
HKDC1	0.744	1.72E−32	0.648	4.72E−06
LGALS3	0.764	3.45E−35	0.611	2.18E−05
LRRC1	0.736	1.98E−31	0.67	1.65E−06
MST1R	0.804	1.92E−41	0.658	2.90E−06
PIK3C2B	0.612	1.52E−19	0.617	1.71E−05
PLEKHA7	0.648	2.00E−22	0.657	3.05E−06
PLS1	0.796	4.54E−40	0.626	1.20E−05
POF1B	0.764	4.29E−35	0.667	1.94E−06
RNF103	0.606	3.86E−19	0.653	3.77E−06
SCNN1A	0.651	1.09E−22	0.619	1.60E−05
SPINT1	0.625	1.50E−20	0.608	2.52E−05
ST14	0.729	1.36E−30	0.612	2.12E−05
TJP3	0.783	6.46E−38	0.747	2.08E−08
TMC5	0.756	4.44E−34	0.652	3.92E−06
TMEM62	0.604	5.43E−19	0.622	1.42E−05
TRIM31	0.665	5.67E−24	0.615	1.90E−05
TSPAN15	0.782	9.99E−38	0.614	1.95E−05
VAMP8	0.611	1.84E−19	0.654	3.50E−06
LIPH	1	0.00E+00	1	0.00E+00

Cor: Pearson correlated coefficient > 0.6 or < −0.6, $P < 0.05$

TAMs, Treg cells and Th2/Th1 in PC were observed in both TCGA PC dataset and GES62452 datasets. Of note, we found LIPH might be involved in the MST1 signaling pathway, which further supported that high expression of LIPH significantly associated with high infiltration of TAMs. Bayne et al. reported that KRAS mutated PC cells secret granulocyte–macrophage colony-stimulating factor, recruiting myeloid-derived suppressor cell and impairing the antitumor activity of $CD8^+$ T cells [41]. Early studies have also reported that KRAS inhibits the expression of components of the antigen presentation pathway, allowing the evasion of TILs [42]. Our study demonstrated that KRAS mutation significantly upregulated the expression of LIPH, and LIPH overexpression was significantly correlated with KRAS activation (Cor = 0.5, $P < 0.05$ in TCGA PC dataset; Cor = 0.58, $P < 0.05$ in GSE62452 dataset; Cor = 0.51, $P < 0.05$ in cBioportal database) (Fig. 4C, E, and S3A). Taken together, we proposed that LIPH overexpression and KRAS activation cooperated with each other to induce immune suppression or evasion in PC.

Previous study by Bellone et al. showed that TGF-β is elevated in PC cell lines [43]. TGF-β is reported to suppress tumor formation through blocking cell cycle progression [44, 45]. But the tumor suppressive effect of TGF-β is often inhibited in PC due to the inactivation of TGF-β signaling mediator, SMAD4 [46]. In this study, we observed that LIPH was notably upregulated by SMAD4 mutation in PC. Furthermore, LIPH upregulation was significantly correlated with SMAD4 downregulation (Cor = −0.44, $P < 0.05$ in the TCGA dataset; Cor = −0.42, $P < 0.05$ in the GSE62452 dataset; Cor = −0.43, $P < 0.05$ in cBioportal database) (Fig. 4A3, 4B2, and S1B). Thus, we postulated that there was a bidirectional regulation between LIPH and SMAD4. It has been reported that SMAD4 inactivation promotes KRAS-mediated tumor progression in PC [47]. Moreover, it was reviewed that KRAS mutation in PC increases TGF-β expression, promoting Treg cells recruitment and TAMs polarization and contributing to immunosuppression in the TME [48, 49]. TGF-β could also upregulate Treg cells through switch Th1/Th2 [50]. And the expression of LIPH was positively associated with the infiltration of Treg cells and Th2/Th1. Treg cells and Th2/Th1 have been reported to be strongly associated with poor prognosis and negatively correlated to the presence of $CD8^+$ T cells in PC [51, 52]. In the study by Whiteside et al., Treg cells produce IL-10 and TGF-β, causing the impairment of $CD8^+$ T cells [53, 54]. Bellone G et al. reported that the function of $CD8^+$ T cells in PC patients is impaired when Th2 dominates in the TME [43]. Thus, higher infiltration of Treg cells and Th2/Th1 reflected an immunosuppressive status in the TME. Taken together, we proposed that LIPH overexpression upregulated the infiltration of pro-tumor immune cells, such as TAMs, Tregs, and Th2 cells, through KRAS-SMAD4-TGF-β signaling pathway.

Function enrichment analysis demonstrated that LIPH mediated the EMT signaling pathway and the cell–cell adhesion in PC (Fig. 5B). Li et al. reported that LIPH promoted the progression of papillary thyroid carcinoma through EMT signaling pathway [14]. It was reviewed that EMT played a critical role in tumor immunosuppression [55]. The activation of EMT impairs the therapeutic effects of ICIs [56, 57].

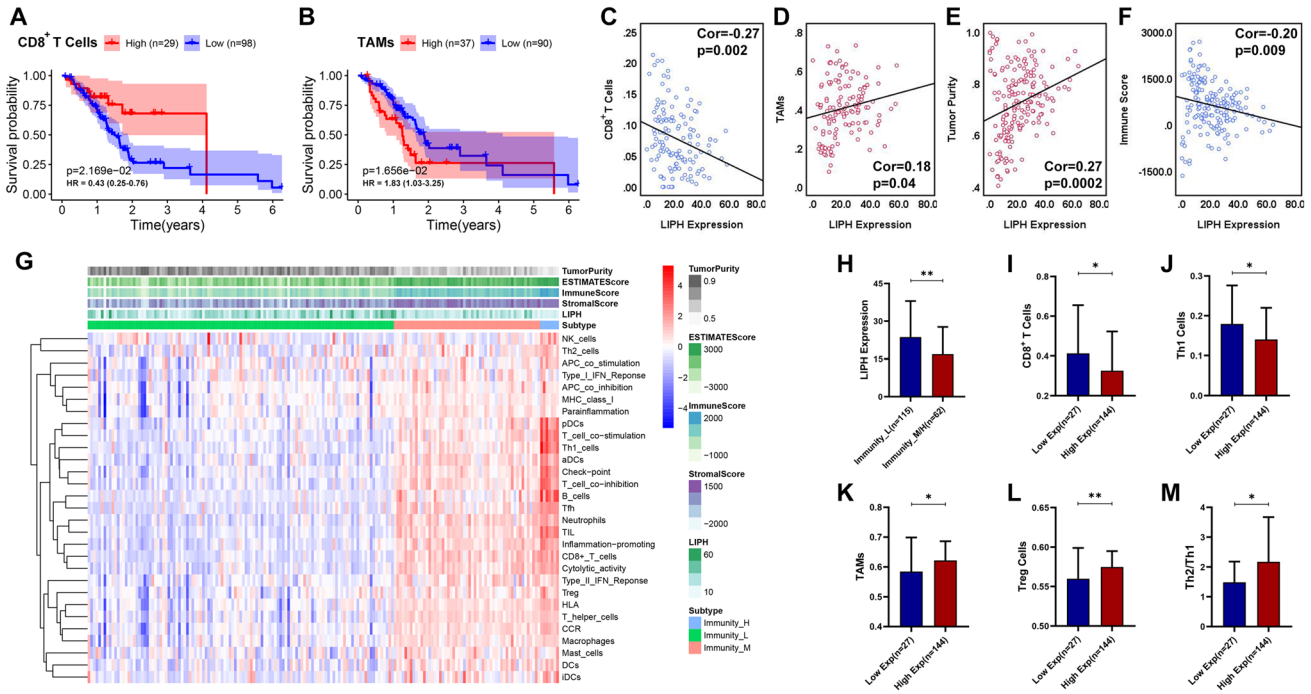

Fig. 6 Association between LIPH expression and the immune infiltration within tumors in TCGA dataset. **A** KM survival analysis showed that patients with lower infiltration levels of $CD8^+$ T cells had a short OS than those with higher infiltration levels of $CD8^+$ T cells ($P<0.05$). **B** Patients with higher infiltration of macrophages had a shorter OS ($P<0.05$) than those with lower infiltration of TAMs ($P<0.05$). **C** LIPH expression negatively correlated with the infiltration level of $CD8^+$ T cells (Cor = −0.27, $P<0.01$). **D** LIPH expression positively correlated with the infiltration level of TAMs (Cor = 0.18, $P<0.05$) and **E** tumor purity (Cor = 0.27, $P<0.001$). **F** LIPH expression negative correlated with immune score (Cor = −0.20, $P<0.01$). **G** Based on the ssGSEA analysis, the enrichment scores of 29 immune-related term were obtained and 177 PC samples in TCGA PC dataset were divided into the immunity L ($n=115$), immunity M ($n=55$), and immunity H ($n=7$) groups. **H** LIPH expression was significantly upregulated in the immunity-L group compared with the immunity-M/H group. **I** LIPH overexpression was significantly associated with low infiltration levels of $CD8^+$ T cells. **J** LIPH overexpression was significantly associated with low infiltration levels of Th1 cells, **K** high infiltration of TAMs, **L** Treg cells and **M** Th2/Th1. (*$P<0.05$; **$P<0.01$;***$P<0.001$; ****$P<0.0001$). TAMs, tumor-associated macrophages; Treg cells, regulatory T cells; Th1, type-1 T helper cells; type-2 T helper cells; ssGSEA, single sample gene set enrichment analysis; KM, Kaplan–Meier; OS, overall survival; Cor, pearson correlated coefficient

With respect to cell–cell adhesion in PC, it was reported that targeting focal adhesion kinase increases PC responsive to checkpoint immunotherapy [40]. Taken together, we supposed that targeting LIPH in PC also enhanced the effect of ICIs. All these results provided an initial understanding about the role of LIPH in immune suppression or evasion within tumor, which remains to be proved in future works. And targeting LIPH-induced EMT, TGF-β or cell–cell adhesion may be a potential effective therapeutic strategy in PC.

Our study has some limitations. Firstly, both the sample sizes of the TCGA PC dataset and the GSE62452 dataset were small. Our findings should be validated with a large sample size dataset in future study. Secondly, the potential function of LIPH in pancreatic carcinogenesis and immune infiltration has not been explored in vitro or in vivo. Furthermore, future works should be conducted to prove the hypothesis about the pathway of KRAS-SMAD4-LIPH-TGF-β in the immunosuppressive TME of PC.

Conclusions

In this study, we found that LIPH overexpression might promote tumor progression, and positively associated with the infiltration of TAMs, Treg cells, and Th2/Th1, while negatively associated with the infiltration of $CD8^+$ T cells and Th1 cells for the first time. Therefore, we identified LIPH as a novel unfavorable prognostic biomarker correlated with immunosuppression, and a novel potential therapeutic target

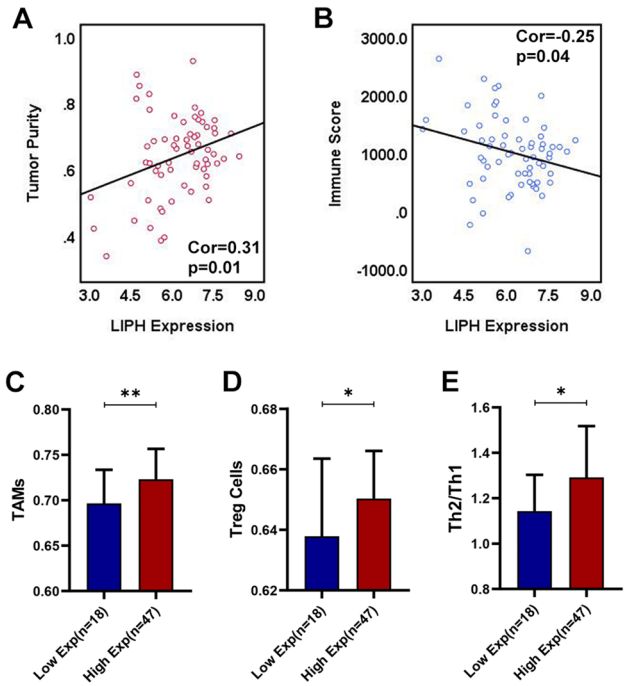

Fig. 7 ESTIMATE algorithm and ssGSEA to validate the association between LIPH expression and immune infiltration within tumors in the GSE62452 dataset. **A** LIPH expression positively correlated with tumor purity (Cor = 0.31, $P = 0.01$) and **B** negative correlated with immune score (Cor = −0.25, $P = 0.04$). **C** High LIPH expression was significantly associated with high infiltration of TAMs, **D** Treg cells and **E** Th2/Th1. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$). TAMs, tumor-associated macrophages; Treg cells, regulatory T cells; Th1, type-1 T helper cells; type-2 T helper cells; ssGSEA, single sample gene set enrichment analysis; Exp, expression; Cor, Pearson correlated coefficient

in PC. This study provides new insights into the tumor-immune microenvironment and immune-based therapies for PC.

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Author contributions HZ, BC, CZ, and BH conceived and designed the study. HZ, XC, and YW performed the data analysis. HZ, XC, BC, and SH wrote the paper. All authors read and approved the manuscript.

Data availability statement The authors confirm that the data supporting the findings of this study are available within the article and its supplementary.

Declarations

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical standards All datasets are freely available as public resources. Therefore, local ethics approval was not needed.

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