Journal of Clinical and Translational Hepatology **2022** vol. 10(1) | 90–103
DOI: 10.14218/JCTH.2021.00047

Original Article

A Novel Prognostic Biomarker LPAR6 in Hepatocellular Carcinoma via Associating with Immune Infiltrates

Jian He*, Mei Meng and Hui Wang*

State Key Laboratory of Oncogenes and Related Genes, Center for Single-Cell Omics, School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Received: 2 February 2021 | **Revised:** 30 March 2021 | **Accepted:** 11 April 2021 | **Published:** 17 September 2021

Abstract

Background and Aims: LPAR6 is the most recently determined G protein-coupled receptor of lysophosphatidic acid, and hardly any study has demonstrated the performance of LPAR6 in cancers. We sought to clarify the relationship of LPAR6 to prognosis potential and tumor infiltration immune cells in different cancers. ***Methods:*** The expression of LPAR6 and its clinical characteristics were evaluated on various databases. The association between LPAR6 and immune infiltrates of various types of cancer were investigated via TIMER. ***Results:*** We determined that higher LPAR6 expression level was associated with a better overall survival. Additionally, high LPAR6 expression level was significantly associated with better disease-specific survival (DSS) in bladder cancer, and better overall survival (OS)/ progression-free survival (PFS)/ distant metastasis-free survival (DMFS)/ relapse-free survival (RFS) in breast cancer and some other types of cancers. Moreover, LPAR6 significantly affects the prognosis of various cancers via The Cancer Genome Atlas (TCGA). Further research exposed that the mRNA level of LPAR6 was positively coordinated with infiltrating levels of devious immune cells in hepatocellular carcinoma. ***Conclusions:*** Our results imply that LPAR6 is associated with prognosis potential and immune infiltration levels in liver cancer. Moreover, LPAR6 expression possibly contributes to the activation of CD8+ T, naive T, effector T cells and natural killer cells and inactivates T regulatory cells, decreases T cell exhaustion and regulate T helper cells in liver cancer. These discoveries imply that LPAR6 could be a novel biomarker of prognosis for indicating prognosis potential and immune-infiltrating level in hepatocellular carcinoma.

Citation of this article: He J, Meng M, Wang H. A Novel Prognostic Biomarker LPAR6 in Hepatocellular Carcinoma via Associating with Immune Infiltrates. J Clin Transl Hepatol 2022;10(1):90–103. doi: 10.14218/JCTH.2021.00047.

Introduction

Hepatocellular carcinoma (HCC) as a kind of malignant tumor that leads to very high morbidity and mortality around the world, especially in China.[1] First-line conventional therapies, such as chemotherapy, radiotherapy and surgery, have demonstrated limited results, and the prognosis of patients remains quite poor. Moreover, recurrence is the main cause of the disease. In the early stages of HCC, surgery is still the main treatment option, while in the later stages, surgery is typically combined with platinum-based adjuvant therapy and cytotoxic chemotherapy and/or radiotherapy.

Immune biology plays a crucial part in oncogenesis and development, and immunotherapy is deemed to be a promising direction of cancer treatment,[2] whereby researchers are trying to modulate the body's own immune system to fight and prevent various type of cancers.[3] In recent years, immunotherapies including monoclonal antibodies and adoptive cell transfers have been increasingly integrated into the clinic for the treatment of various types of cancer, such as melanoma and lung cancer.[4] In the past decade, the discovery of antibodies against immune checkpoints (i.e. PD-1 and PD-L1) have remodeled the treatment of non-small cell lung cancer (NSCLC).[4] Immunotherapy, as represented by PD-1 and PD-L1 inhibitors, has showed promising anti-tumor effects in various types of cancer, including non-small cell lung cancer and melanoma.[5] Anti-CTLA4 also showed a clinical curative effect in HCC, similar to blockade of PD-1 or PD-L1 having shown partial response in advanced liver cancer.[6] Additionally, more and more studies have demonstrated that the tumor-infiltrating

Keywords: LPAR6; TILs; Prognosis; Hepatocellular carcinoma; Biomarker.

Abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; CI, confidence interval; CNS, central nervous system; COAD, colon adenocarcinoma; DC, dendritic cell; DFS, disease-free survival; DLBC, lymphoid Neoplasm Diffuse Large B-cell Lymphoma; DMFS, distant metastasis-free survival; DMSF, distant metastasis-free survival; ESCA, esophageal carcinoma; FDR, false discovery rate; GPCR, G protein-coupled receptor; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; HR, hazard ratio; HNSC, head and neck cancer; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NK, natural killer cells; NSCLC, non-small cell lung cancer; OS, overall survival; OV, ovarian serous cystadenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PFS, progression-free survival; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; RFS, relapse-free survival; SARC, Sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TAMs, tumor-associated macrophages; TCGA, The Cancer Genome Atlas; Tfh, follicular helper T; TGCT, Testicular Germ Cell Tumors; Th, T-helper; THCA, thyroid carcinoma; THYM, Thymoma; TIICs, tumor-infiltrating immune cells; TILs, tumor-infiltrating lymphocytes; TINs, tumor-infiltrating neutrophils; TME, tumor microenvironment; Tregs, T regulatory cells; UCEC, uterine corpus endometrial carcinoma; UVM, Uveal Melanoma.

***Correspondence to:** Hui Wang and Jian He, State Key Laboratory of Oncogenes and Related Genes, Center for Single-Cell Omics, School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China. ORCID: https://orcid.org/0000-0002-1426-7799 (JH). Tel/Fax: +86-21-6384-6590, E-mail: huiwang@shsmu.edu.cn (HW), jih003@sjtu.edu.cn (JH)

lymphocytes (TILs) play an important role in response to chemotherapy and improving prognosis of various types of cancer,[7] such as tumor-associated macrophages (TAMs)[8] and tumor-infiltrating neutrophils (TINs), which also contribute to prognosis.[9,10] However, the bull's-eye for HCC treatment via targeting tumor microenvironment (TME) remains unelucidated. It has been demonstrated that the hepatic microenvironment epigenetically shapes lineage commitment in mosaic mouse models of liver tumorigenesis.[11] TME of HCC harbors a significant level of T cells, as indicated by small conditional RNA-sequencing, however, and the TILsare incapable of killing tumor cells,[12] implying that TME is very complicated. So, there is an urgent demand to clarify the immunophenotype of tumor-immune interactions and identify new immune-related therapeutic targets for liver cancer.

Lysophosphatidic acid (LPA) is a kind of lipid that is involved in tumor proliferation and one of their receptors (LPAR6) is the latest determinate G protein-coupled receptor (GPCR) of LPA family,[13,14] and it has been revealed to be associated with several types of cancer, including colorectal, prostate, pancreatic cancer and HCC.[15–18] However, the function of LPAR6 remains highly controversial, as demonstrated by the previous studies. LPAR6 acts as a tumor suppressor and inhibits tumor migration in colorectal cancer, whereas in the other tumors mentioned, the LPAR6 protein might act as a facilitator.[16–18] All these findings indicate that the proteins encoded by LPAR6 may play an essential role in cancer, but the association between LPAR6 and tumor progression and the underlying mechanism is still not well understood.

Our previous study showed that LPAR6 was highly expressed in several T cell subgroups, including naïve T cells, CD38+ T cells and plasmablasts, while showing low expression in T regulatory cells (Tregs) and exhausted T cells in chemical-induced cancer mouse model (unpublished data), based on single-cell RNA sequencing. These findings suggest that LPAR6 may have multifaceted functional roles in modulating or recruiting TILs; in this way, they are able to remodel the tumor microenvironment. However, the underlying functions and mechanisms of LPAR6 in tumor progression and tumor immunology is still not well understood.

In this work, we extensively studied the expression level of LPAR6 and the relationship with prognosis of cancer patients according databases as Oncomine, PrognoScan, and Kaplan-Meier Plotter. Moreover, we investigated the correlation of LPAR6 with tumor-infiltrating immune cells in the different tumor microenvironments.

All the findings in this report throw the light on the key role of LPAR6 in HCC and provide insight into a potential relationship and a fundamental mechanism between LPAR6 and tumor-immune interactions.

Methods

Gene expression level determination of LPAR6

The gene expression level of the LPAR6 in various types of cancers was determined via Oncomine,[19] as we previously described.[20] The threshold was set as follows: *p*-value of 1E-6, fold-change of 2, and gene ranking top 5%.

PrognoScan database determination

The relationship between LPAR6 expression and survival in various types of cancers was determined by PrognoScan and GEPIA2.[21,22] The threshold was adjusted to a Cox *p*-value of <0.05.

Correlation analysis

The correlation between LPAR6 expression and survival rate as well as different cancer staging in various cancers was analyzed by the Kaplan-Meier Plotter.[23] The hazard ratio (HR) with 95% confidence intervals (CIs) and log-rank *p*-values were also computed.

Methylation analysis

UALCAN[24] was used to analyze methylation and relative expression level of LPAR6, as well as the survival of a target gene across several clinicopathological characteristics. The t-test was performed to determine statistical significance.

GeneMANIA analysis

GeneMANIA identified single genes associated to a set of input genes[25] and was used to construct the LPAR6 biological network based on a set of functional association data, including co-expression, genetic and protein interaction pathways, co-localization and protein domain homology.

LinkedOmics analysis

Thirty-two types of cancer and over 10,000 patients from The Cancer Genome Atlas (TCGA) were included in the LinkedOmics database.[26] LinkFinder was employed to determine the differentially expressed genes. LUAD and LUSC cohorts whose expression levels correlated with those of LPAR6. LinkInterpreter was employed to identify the pathways and networks.[27]

Immune infiltrates level and gene correlation analysis

We determined the expression level of LPAR6 in various types of cancer and the association of LPAR6 expression with the abundance of immune-infiltrating cells, including CD4+ T, CD8+ T and B cells, macrophages, neutrophils and dendritic cells via gene modules in TIMER.[28] In addition, all these correlations between LPAR6 expression and gene markers of tumor-infiltrating immune cells (TIICs) were explored. The gene markers of TIICs included markers of T cells (CD8+ T and general T cells), monocytes, B cells, tumor-associated macrophages (TAMs), macrophages (M1 and M2), neutrophils, natural killer cells (NK), dendritic cells (DCs), follicular helper T (Tfh) cells, T-helper (Th1, Th2 and Th17) cells, Tregs, and exhausted T cells. The gene marker sets have been mentioned in our former studies.[29] The expression level of the genes were demonstrated by using log2 RSEM (RNA-seq by expected maximization).

The GEPIA2 database was employed to confirm the significantly correlated genes in-depth; this is a web server with gene expression interpretation based on TCGA and the GTEx databases.[22] Furthermore, GEPIA2 was employed to produce curves of overall survival (OS) and disease-free survival (DFS). The Spearman procedure was employed to analyze the correlation coefficient.

Statistical analysis

The statistical analysis was conducted as in our previous work. The results produced via Oncomine are exhibited with *p*-values and fold-changes. The consequence of PrognoScan, Kaplan-Meier plots and GEPIA are exhibited with HR and *p*/Cox *p*-values. The correlation coefficient of gene expression was analyzed and *p*-values <0.05 were regard as statistically significant. The Kaplan-Meier plot and corresponding log-rank test were used to evaluate the differences in OS between the groups.

Results

mRNA expression levels of LPAR6 in different types of human cancers

To determine differences of LPAR6 expression in tumor and normal tissues, the LPAR6 mRNA levels in different tumors and normal tissues of multiple cancer types were analyzed using the Oncomine database. This analysis revealed that the LPAR6 expression was higher in kidney cancer, leukemia, liver cancer and lymphoma compared to the normal tissues, and lower expression of LPAR6 was observed in bladder cancer, breast cancer, cervical cancer and esophageal cancer compared to the normal tissues (cancer vs. normal) (Fig. 1A). In addition, LPAR6 expression was higher in brain and central nervous system (CNS), leukemia, ovarian cancer and sarcoma in tumor tissues (cancer vs. cancer), whereas in brain and CNS, kidney, ovarian cancer and sarcoma, LPAR6 is low expression (Fig. 1A). The detailed results of LPAR6 expression in different cancer types are summarized in Supplementary Table 1.

To further evaluate LPAR6 expression in human cancers, we examined LPAR6 expression using the RNA-sequencing data of multiple malignancies in TCGA. The differential expression between the tumor and adjacent normal tissues for LPAR6 across all TCGA tumors is shown in Figure 1B. LPAR6 expression was significantly lower in the tumor tissue of bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), head and neck cancer (HNSC), kidney chromophobe (KICH), lung adenocarcinoma (LUAD), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ) and uterine corpus endometrial carcinoma (UCEC) compared with adjacent normal tissues and was significantly higher in esophageal carcinoma (ESCA), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), thyroid carcinoma (THCA) compared with adjacent normal tissues and slightly lower in liver hepatocellular carcinoma (LIHC) tumor tissue (Fig. 1 B).

GEPIA2 generates dot plots profiling gene/isoform expression across multiple cancer types and paired normal adjacent samples. The differential expression level for LPAR6 between tumor and matched TCGA normal data across all TCGA is shown in Figure 1C. The expression level of LPAR6 was significantly higher in cholangiocarcinoma (CHOL), ESCA and KIRC and lower in KICH, BRCA, KICH and UCEC compared with the adjacent normal tissues, and slightly higher in LIHC tumor tissue.

Prognostic potential of LPAR6 in cancers

We investigated whether LPAR6 level was associated with various types of cancer. The relationships between LPAR6 expression and prognosis of different cancers are shown in Supplementary Table 2. Notably, LPAR6 expression significantly affects OS in several types of cancer, including lung, bladder, breast, colorectal, eye and ovarian (Fig. 2A–M). Notably, LPAR6 expression significantly impacts OS in two types of cancers, namely lung and breast (Fig. 2A, B, D). Two cohorts (GSE3141 and GSE4573) of lung cancer showed that high LPAR6 expression was associated with better prognosis (OS HR=0.53, 95% CI=0.36 to 0.80, Cox *p*=0.00206181; OS HR=0.53, 95% CI=0.31 to 0.91, Cox *p*=0.0219869) (Fig. 2A, B). Therefore, it is conceivable that higher LPAR6 expression is an independent risk factor and leads to a better prognosis in lung cancer patients, and HR below 0 indicates LPAR6 expression is a protective factor. Also, high LPAR6 expression significantly impacts DSS in bladder cancer and RFS and DFS in the breast cancer (Fig. 2C, E, F). Moreover, three cohorts (GSE19615, GSE9195 and GSE11121) of breast cancer showed that the higher LPAR6 expression was associated with a better prognosis of DMFS (Fig. 2G–I). Higher expression level of LPAR6 was associated with better prognosis in some other types of cancer (Fig. 2J–M).

To further explore the prognostic potential of LPAR6 in various types of cancer, the Kaplan-Meier Plotter database was employed to determine the LPAR6 prognostic value based on Affymetrix microarrays and RNA-sequencing data. Similarly, better prognosis in breast cancer (OS and DMSF) and lung cancer (OS and PPS) was shown to correlate with higher LPAR6 expression (Fig. 2N–P, T–V). Better prognosis (OS, PFS and RFS) in liver cancer was shown to correlate with higher LPAR6 expression level (Fig. 2Q–S). These data supported the prognostic value of LPAR6 in some specific types of cancers and that increased and decreased LPAR6 expression have different prognostic values depending on the specific cancer type.

The RNA-sequencing data in the TCGA were also used to explore the prognostic potential of LPAR6 in different cancers via GEPIA2. We analyzed the association between LPAR6 expression and prognostic values in 33 types of cancer. The expression level of LPAR6 significantly impacts prognosis in three types of cancers, including adrenocortical carcinoma (ACC), lower grade glioma (LGG) (Supplementary Fig. 1). High LPAR6 expression levels were associated with better prognosis of OS in skin cutaneous melanoma (SKCM) but appeared to have less influence on DFS. These results confirmed the prognostic value of LPAR6 in some specific types of cancers and that decreased and increased LPAR6 expression have different prognostic values depending on the type of cancers.

High LPAR6 expression impacts the prognosis of liver cancer in different clinical characteristics

To better study the relevance and underlying mechanisms of LPAR6 expression in cancer, we investigated the correlation between the LPAR6 expression level and clinical characteristics of liver cancer, especially the different clinical stages.

High expression of LPAR6 was correlated with better OS in the early stage of the progress of cancer (Table 1). Higher expression of LPAR6 was correlated with better OS in both female and male patients, whereas different association patterns were shown among different races (Table 1). Besides the Asian race, LPAR6 was associated with better OS in people of the White race.

Higher LPAR6 expression level was associated with better OS in stage 1 and 1+2 and better PFS in stage 1+2 of HCC patients respectively but was not correlated with OS and PFS of other stages. This phenomenon was also verified by the American Joint Committee on Cancer classification, we found that higher expression of LPAR6 was associated with better OS only in HCC early stage (OS HR=0.5, *p*=0.024) (Table 1). The vascular invasion could also function as an indicator of cancer staging during progression.[30] We discovered that higher LPAR6 expression was correlated with better OS in the non-vascular invasion liver cancer patients.

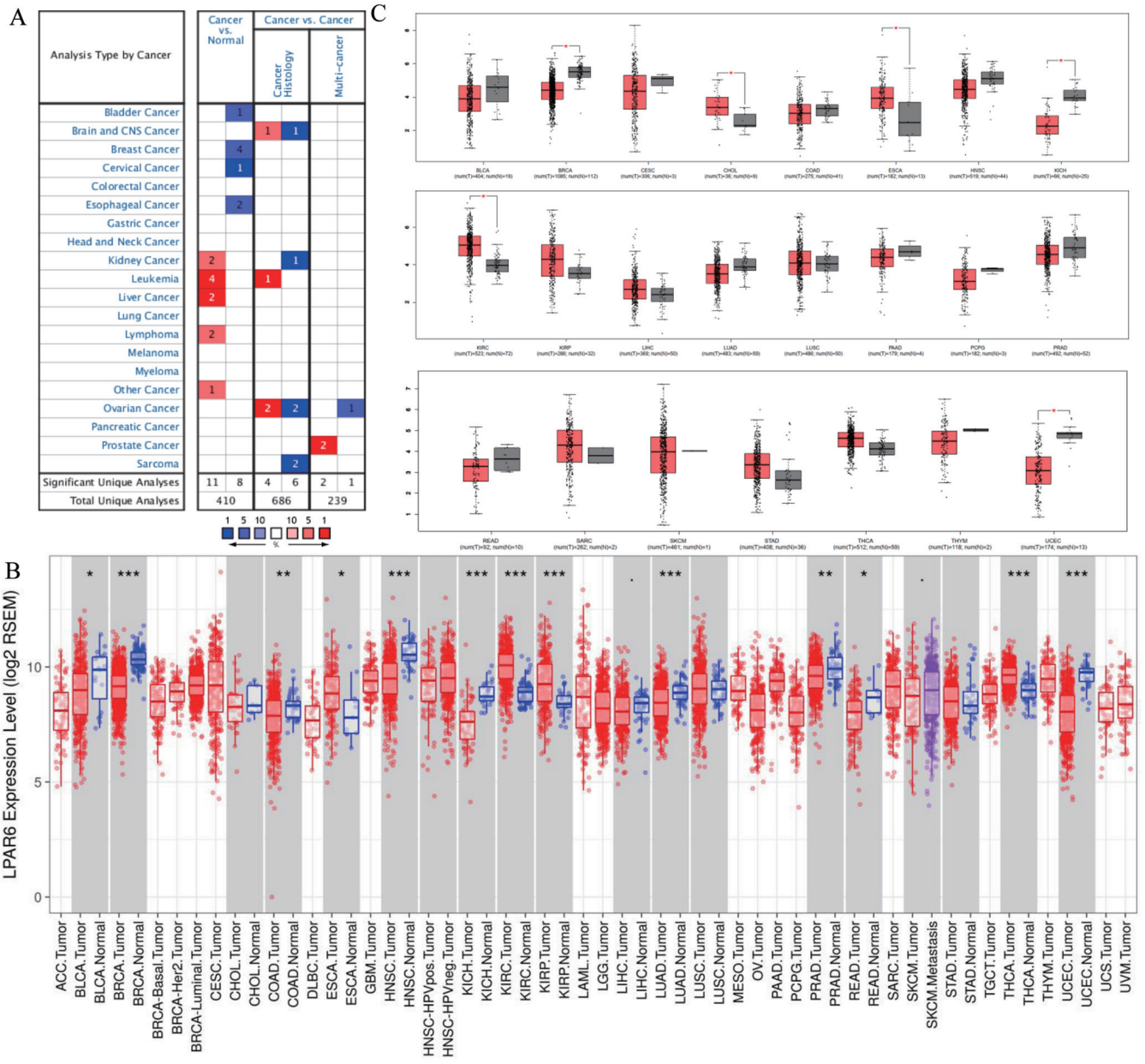

Fig. 1. *LPAR6* mRNA expression levels in different types of human cancers in different databases. (A) Increased or decreased LPAR6 in datasets of different cancers compared with normal tissues in the Oncomine database. Cell color is determined by the best gene rank percentile for the analyses within the cell. (B) Human LPAR6 expression levels in different tumor types from the TCGA database. *p*-value significance codes: 0≤ ***<0.001 ≤ **<0.01 ≤ *<0.05 ≤ .<0.1. The threshold was set as follows: *p*-value of 1E-6, fold-change of 2, and gene ranking of top 5%. (C) LPAR6 expression profile across all tumor samples and paired normal tissues are shown in dot plot. Each dot represents the expression of samples. The gene expression profile across all tumor samples and paired normal tissues are shown in bar plot. The height of bars represents the median expression of certain tumor types or normal tissue (each dot representing a distinct tumor or normal sample). TCGA, The Cancer Genome Atlas.

All these consequences suggest that LPAR6 expression level can affect the prognosis in early HCC staged patients but not associated with PFS and OS of late stage HCC patients.

Low promoter methylation levels of LPAR6 impacts the clinicopathological parameters of liver cancer patients

Low promoter methylation levels of LPAR6 were associated with the earlier stage of the progress of cancer in LIHC (Fig. 3), which implies that the earlier stage, the lower promoter methylation levels of LPAR6. This could be an explanation to the higher LPAR6 expression level being associated with better OS in earlier stage HCC (Fig. 3).

Interaction network of LPAR6

We found that LPAR6 co-expressed with 19 proteins, and

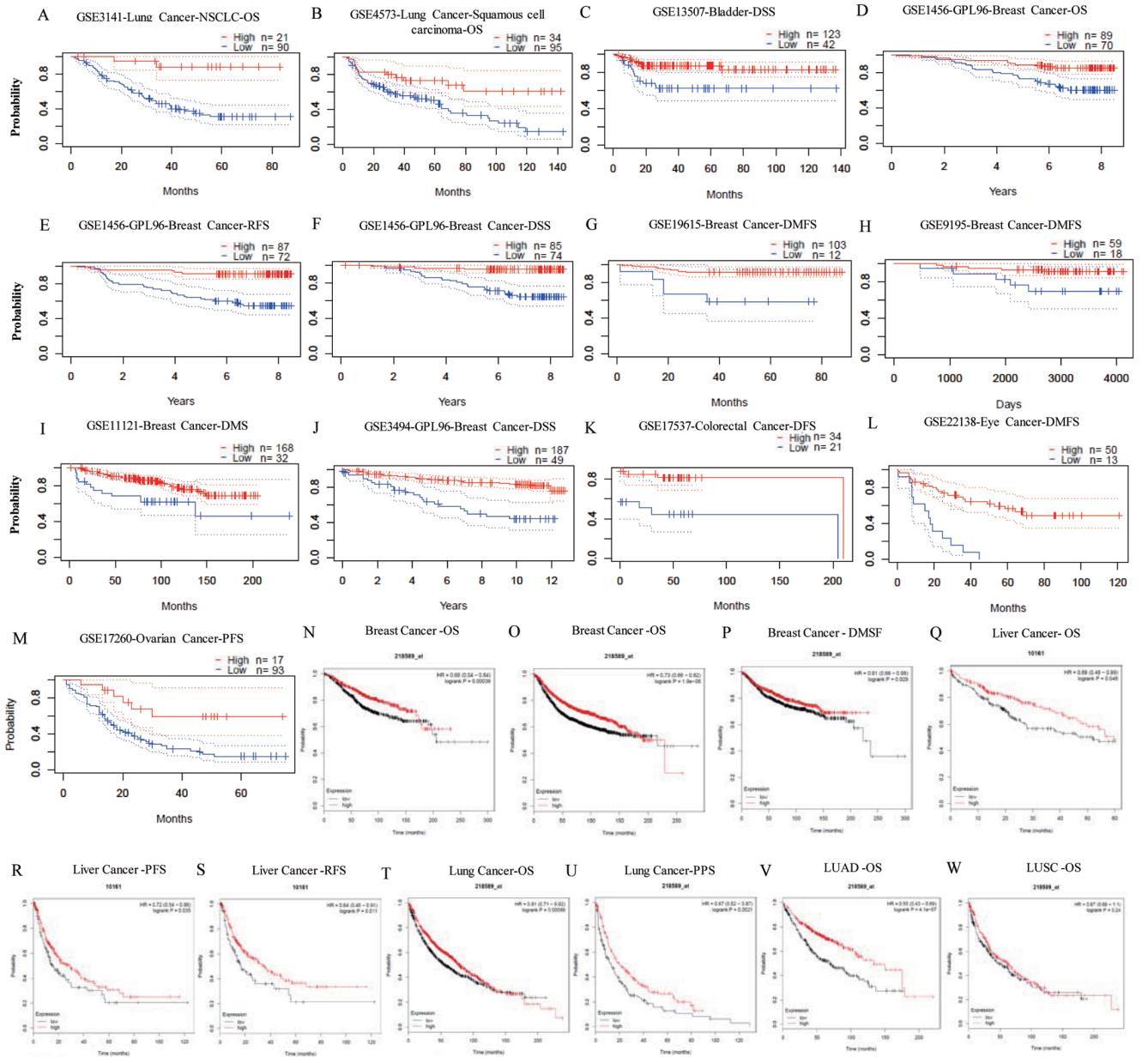

Fig. 2. Kaplan-Meier survival curves comparing the high and low expression of LPAR6 **in different types of cancer in the PrognoScan (A–M) and Kaplan-Meier Plotter databases (N–W).** (A–C) Survival curves of OS in two lung cancer cohorts [GSE3141 (n=111, p=0.00206181) and GSE4573 (n=129, p=0.0219869)] and DSS in the bladder cancer cohort [GSE13507 (n=165, p=0.0067285)]. (D–F) Survival curves of OS, RFS and DFS in the breast cancer cohort [GSE1456-GPL96 (n=159, p=0.00575883; p=0.0000252; p=0.000210173)]. (G–I) Survival curves of DMFS in the breast cancer cohort [GSE19615 (n=159, p=0.00575883), GSE9195 (n=159, p=0.0466683), GSE11121 (n=200, p=0.0389008)]. (J–L) Survival curves of DSS in breast cancer cohort, DFS in colorectal cancer cohort and DMFS in the eye cancer cohort [GSE3494 (n=236, p=0.00294205), GSE17537 (n=55, p=0.0257972), GSE22138 (n=63, p=0.00092478)]. (M) Survival curves of PFS in the ovarian cancer cohort [GSE17260 (n=110, p=0.0392865)]. (N–P) Survival curves of OS (n=1402), RFS (n=3951) and DMSF (n=1746) in the breast cancer cohort. (Q–S) Survival curves of OS (n=364), FPS (n=370) and RFS (n=316) in the liver cancer cohort. (T, U) Survival curves of OS (n=1926) and PPS (n=344) of the lung cancer cohort. (V,W) Survival curves of OS of the lung adenocarcinoma cohort (n=720) and squamous cell carcinoma cohort (n=524). OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; DMFS, distant metastasis-free survival; PFS, progression-free survival.

shared protein domains with ADRB2 and physical interactions with dystrophin by constructing a LPAR6 interaction network (Fig. 4A). The top 50 negatively [green spot; false discovery rate (FDR) <0.05] and positively correlated genes (red spot; FDR<0.05), with the expression of LAPR6 displayed as a volcano plot by LinkedOmics online tools (Fig. 4B). These results indicate that LPAR6 serves a critical role in cancer development. A strong positive association between the expression levels of LPAR6 was revealed by Pearson's correlation coefficient analysis. Biological process and molecular function analyses showed that LPAR6-associated differentially expressed genes were involved in a number of biological processes and molecular functions, including 'interleukin production', 'cytokine production', 'respiratory burst', 'inflammatory cell apoptotic process', 'inflammatory response', and some other immune biology process in LIHC

Table 1. Correlation of *LPAR6* mRNA expression and clinical prognosis in HCC with different clinicopathological factors by the Kaplan-Meier Plotter

Clinicopathological characteristics	OS, *n*=364			Progression-free survival, *n*=370		
	n	**HR**	***p***	***n***	**HR**	***p***
Sex						
Female	118	0.45 (0.24–0.86)	*0.013*	120	0.75 (0.41–1.35)	0.33
Male	246	0.57 (0.36–0.91)	*0.017*	246	0.66 (0.46–0.95)	*0.024*
Race						
White	181	0.5 (0.3–0.84)	*0.0073*	183	0.7 (0.47–1.06)	0.088
Black or African American	17	–	–	17	–	–
Asian	155	0.68 (0.37–1.25)	0.21	155	0.62 (0.38–1.01)	0.054
Stage						
1	170	0.47 (0.25–0.9)	*0.02*	170	1.55 (0.94–2.55)	0.084
1+2	253	0.6 (0.37–0.97)	*0.037*	254	0.54 (0.36–0.83)	*0.0039*
2	83	0.52 (0.22–1.24)	0.13	84	0.59 (0.32–1.06)	0.075
2+3	166	1.45 (0.85–2.45)	0.17	167	0.76 (0.51–1.14)	0.19
3	83	1.57 (0.85–2.89)	0.14	83	1.28 (0.72–2.28)	0.4
3+4	87	1.57 (0.87–2.84)	0.13	88	1.25 (0.71–2.19)	0.44
4	4	–	–	5	–	–
AJCC_T						
1	180	0.5 (0.27–0.92)	*0.024*	180	1.48 (0.91–2.4)	0.11
2	90	0.56 (0.25–1.26)	0.16	92	0.6 (0.35–1.05)	0.07
3	78	1.65 (0.88–3.1)	0.11	78	1.36 (0.75–2.49)	0.31
4	13	–		13	–	
Vascular invasion						
None	203	0.46 (0.27–0.78)	*0.0034*	204	0.64 (0.4–1.02)	0.06
Micro	90	2.27 (0.95–5.38)	0.057	91	0.7 (0.36–1.38)	0.3
Macro	16	–		16		
Risk factors						
Alcohol consumption						
Yes	115	0.56 (0.29–1.06)	0.07	115	0.59 (0.35–0.98)	*0.041*
None	202	0.58 (0.35–0.94)	*0.026*	204	0.77 (0.51–1.15)	0.2
Hepatitis virus						
Yes	150	0.56 (0.29–1.07)	0.076	152	0.53 (0.33–0.87)	*0.01*
None	167	0.57 (0.34–0.98)	*0.03*	167	0.76 (0.49–1.17)	0.21

Italicized values indicate $p<0.05$. ACC, adrenocortical carcinoma; HR, hazard ratio; OS, overall survival.

(Fig. 4C). All the above imply that LPAR6 serves a key role in immune system activation, cellular responses to stimulation, metabolism and a number of other processes.

LPAR6 expression is correlated with immune infiltration level in HCC

Tumor-infiltrating lymphocytes are an independent predictor of survival in cancers.[31,32] Therefore, we investigated whether LPAR6 expression was correlated with immune infiltration levels in different types of cancer. We assessed the correlations of LPAR6 expression with immune infiltration levels in 39 cancer types from TIMER. The results showed that LPAR6 expression had significant negative correlations with tumor purity in 26 types (BLCA, BRCA, BRCA-Basal, BRCA-Her2, BRCA-Luminal, CESC, CHOL, DLBC, GBM, HNSC, HNSC-HPVneg, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PCPG, SARC, SKCM, SKCM-Metastasis, STAD, TGCT, UCEC, and UVM) of cancer, which indicates LPAR6 is somehow related to recruiting lymphocytes to the tumor, and significant correlations with B cell infiltration levels in 13 types of cancers (BRCA-Basal, BRCA-Luminal, CHOL, COAD, GBM, HNSC-HPVpos, KIRC, LGG, LIHC, LUAD, THCA, THYM, UCEC) (Supplementary Fig. 2). In addition, LPAR6 expression had significant correlations with infiltrating levels of CD8+ T cells in 24 types of cancer (ACC, BRCA, BRCA-Basal, BRCA-Her2, BRCA-Luminal, CESC, COAD, HNSC, HNSC-HPVpos, HNSC-HPVneg, KIRC, KIRP, LGG, LIHC, LUAD, MESO, OV, READ, SKCM, SKCM-Primary,

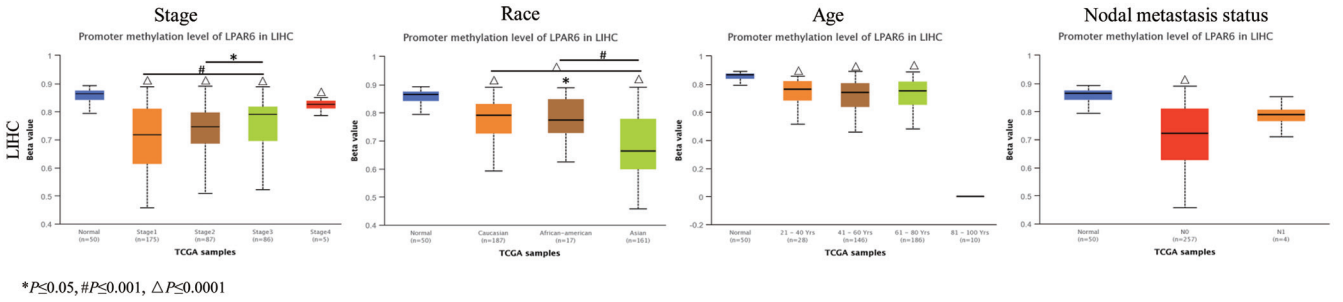

Fig. 3. Promoter methylation levels of *LPAR6* impacts the clinicopathological parameters in LIHC cohorts. LIHC, liver hepatocellular carcinoma.

SKCM-Metastasis, STAD, THCA, UCEC), CD4+ T cells in 26 types of cancer (ACC, BLCA, BRCA, BRCA-Basal, BRCA-Her2, BRCA-Luminal, CESC, CHOL, COAD, DLBC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, MESO, OV, PAAD, PCPG, STAD, TGCT, UCEC, USC), macrophages in 20 types of cancer (ACC, BRCA, BRCA-Basal, BRCA-Her2, BRCA-Luminal, CHOL, COAD, HNSC, HNSC-HPVneg, KIRP, LGG, LIHC, LUAD, MESO, OV, PCPG, SKCM, SKCM-Metastasis, STAD, UCEC), neutrophils in 33 types of cancer (ACC, BLCA, BRCA, BRCA-Basal, BRCA-Her2, BRCA-Luminal, CESC, COAD, ESCA, HNSC, HNSC-HPV-pos, HNSC-HPVneg, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, READ, SKCM, SKCM-Primary, SKCM-Metastasis, STAD, TGCT, THCA, THYM, UCEC, UCS, UVM), and dendritic cells in 21 types of cancer (ACC, BRCA, BRCA-Basal, BRCA-Her2, BRCA-Luminal, CESC, CHOL, COAD, ESCA, KIRC, KIRP, LGG, LIHC, LUAD, OV, PCPG, SKCM, STAD, THCA, THYM, UCEC). (Supplementary Fig. 2).

In view of the correlation between the expression level of LPAR6 and the level of immune infiltration in various types of cancer, we can determine that LPAR6 is associated with prognosis and immune infiltration in specific cancer types. Tumor purity reflects the degree of immune infiltration of clinical tumor samples, and purity is negatively correlated with the degree of immune infiltration.[22,28] Therefore, we selected cancer types in which the expression level of LPAR6 was significantly negatively correlated with tumor purity in TIMER and significantly correlated with prognosis. What attracted our attention is that the expression level of LPAR6 is associated with a better OS prognosis and higher immune infiltration in HCC.

The expression level of LPAR6 in LIHC was significantly negatively correlated with tumor purity (Fig. 5). The expression level of LPAR6 was significantly positively correlated with the infiltration level of T cells (CD8 + T, CD4 + T) B cells, neutrophils, macrophages and dendritic cells in LIHC (Fig. 5). These findings strongly indicate that LPAR6 plays a specific role in immune infiltration in different types of HCC.

Correlation analysis between LPAR6 expression and immune marker sets

In order to study the relationship between LPAR6 and various immune infiltrating cells, we studied the correlation between LPAR6 and the immune marker sets of various immune cells of LIHC in the TIMER and GEPIA databases. We analyzed the correlation of the expression level of LPAR6 of different immune cells (including T cells (CD8 + T and T general), B cells, monocytes, neutrophils, tumor association macrophages, natural killer cells, M1 and M2 macrophages and dendritic cells between immune marker genes' expression in LIHC (Table 2 and Fig. 6). We also analyzed the different functional T cells, such as Th1, Th2, Th17, follicular helper T cells, and Tregs, as well as exhausted T cells.[33]

After correlation adjustment for purity, the results showed that the expression level of LPAR6 was significantly related to most immune marker sets of various immune cells and T cell subtypes, especially the effector T cells in LIHC, which were negatively related to THYM, and THYM was related to poor prognosis. We employed THYM as a negative control here. (Table 2 and Fig. 6).

We found that the expression levels of the marker genes in general T cells, CD8+ T cells, naive T cells, effector T cells, natural killer cells, M1 macrophages and dendritic cells have strong correlations with LPAR6 expression in LIHC (THYM as a negative control which with poor prognosis) (Table 2). Specifically, we showed NOS2, IRF5 and PTGS2 of M1 phenotype were significantly correlated with LPAR6 expression in LIHC ($p<0.0001$). It is reported that M1 could prevent tumor development [8,10]. Further studies need to be done on whether LPAR6 is a crucial factor mediating the de-polarization of macrophages and remodeling the tumor microenvironment. In addition, for Tregs, LPAR6 did not demonstrate a correlation with the Treg markers, such as STAT5B in LIHC (Table 2). We further analyzed the correlation between LPAR6 expression and the above markers of monocytes and various types of T cells in normal and tumor tissue in the GEPIA database, and we found that the correlation results between LPAR6 and markers of monocytes and TAMs were similar to those in TIMER (Supplementary Table 3, Figs. 7, 8).

Different correlation patterns between tumor and normal tissue in LIHC patients

We found that the expression levels of most marker sets of immune cells, including resident memory T cells, effector Treg, Th1-like, have strong correlations with LPAR6 expression both at a similar level in tumor and normal tissue in the LIHC. The more interesting thing is that in the naive T cell, effector T cell, effector memory T cell, central memory T cell and T cell exhaustion populations, the correlation coefficients were higher in normal tissue, whereas an inverse phenomenon had been detected in the resting Treg population (Fig. 8 and Supplementary Table 3). High LPAR6 expression relates to a high infiltration level of dendritic cells in the tumor tissue of LIHC patients, dendritic cells markers such as HLA-DQB1, CD1C and NRP1 show significant correlations with LPAR6 expression both in the tumor tissue in LIHC (Supplementary Table 3). HLA-DPB1, HLA-DRA, HLA-DPA1 and CD11c also showed significant correlations with LPAR6 expression in both tumor and normal tissue in LIHC (Supplementary Table 3 and Fig. 8). These results further revealed that there is a strong relationship between LPAR6 and dendritic cell infiltration. This finding suggests that there are different correlation patterns between tumor and normal tissues in LIHC patients. This exciting finding indi-

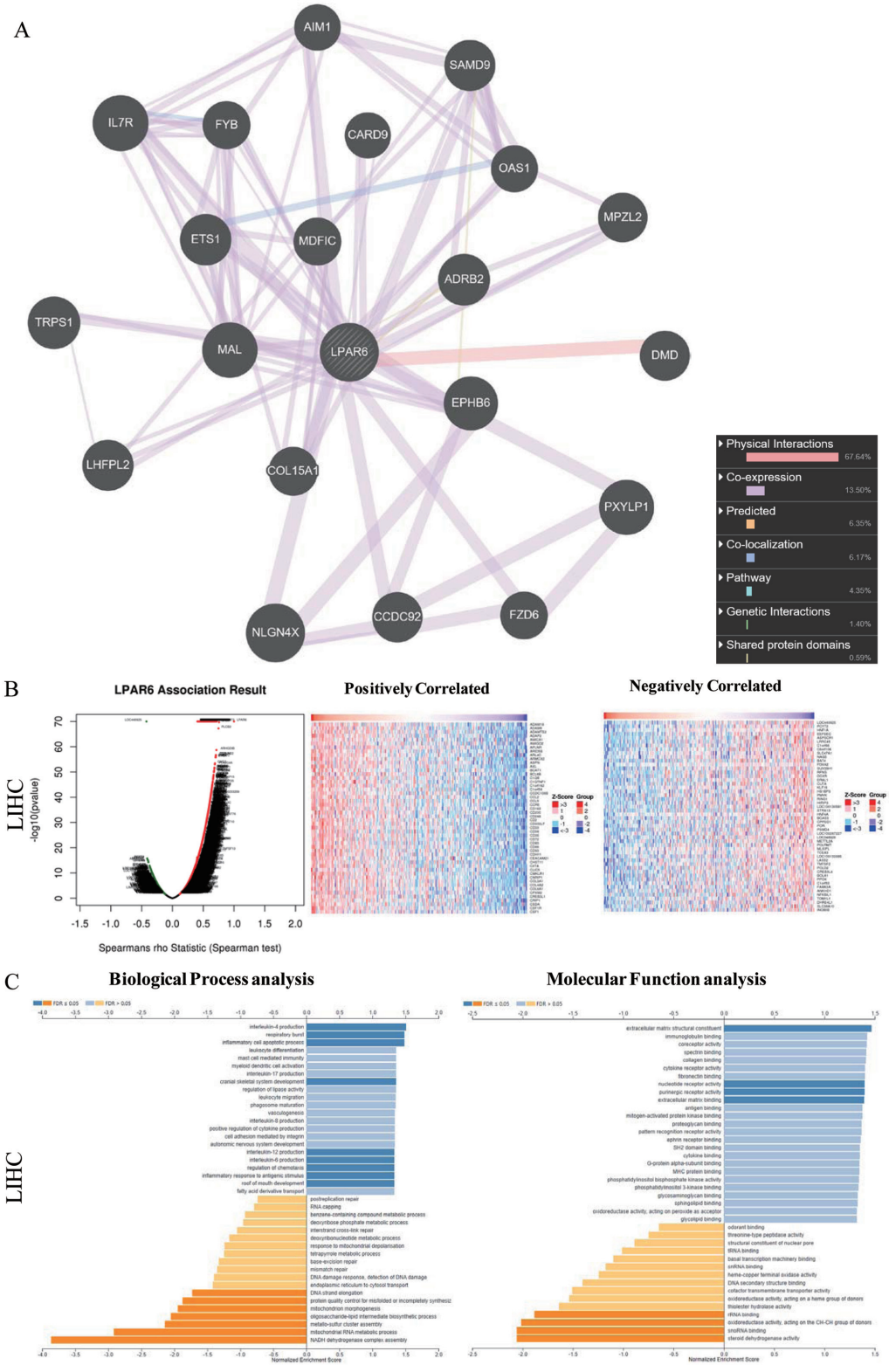

Fig. 4. Biological interaction network and enriched gene ontology annotations of *LPAR6* correlated genes in LIHC. LPAR6 interaction network in TCGA. Different colors represent diverse bioinformatics methods (A) and differentially expressed genes in correlation with LPAR6. Heat maps of positively and negatively correlated genes with LPAR6 in LIHC were analyzed by Pearson's test (B). Red indicates positive and blue indicates negative. Enriched Gene Ontology annotations of Biological Process and Molecular Function analysis of LPAR6 correlated genes in LIHC (C). Dark blue and orange indicate FDR ≤0.05, light blue and orange indicate FDR >0.05. FDR, false discovery rate. LIHC, liver hepatocellular carcinoma; TCGA, The Cancer Genome Atlas; LIHC, liver hepatocellular carcinoma; FDR, false discovery rate.

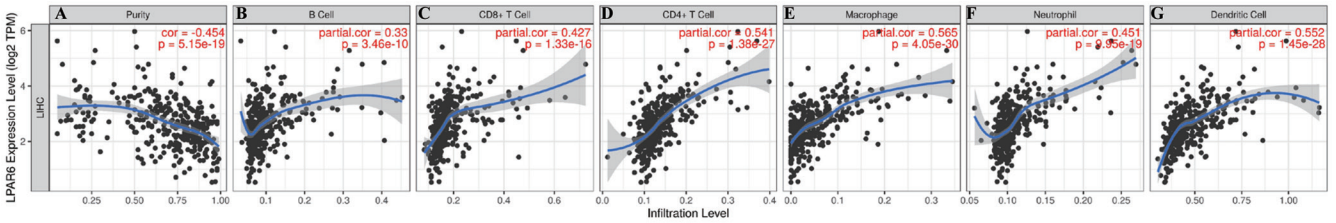

Fig. 5. Correlation of *LPAR6* expression with immune infiltration level in HCC. LPAR6 expression is significantly negatively related to tumor purity (A). LPAR6 expression has significantly strong positive correlations with the level of B cells (B), CD8+ T cells (C), macrophages (E), neutrophils (F), and dendritic cells (G). HCC, hepatocellular carcinoma.

Table 2. Correlation analysis between *LPAR6* and related marker genes of immune cells

Description	Gene markers	LIHC				THYM			
		None		Purity		None		Purity	
		Cor	*p*	Cor	*p*	Cor	*p*	Cor	*p*
CD8+ T cell	CD8A	0.505	***	0.341	***	−0.108	0.239	−0.177	0.0588
	CD8B	0.451	***	0.292	***	−0.219	0.0162	−0.291	*
T cell, general	CD3D	0.474	***	0.312	***	−0.163	0.0745	−0.24	*
	CD3E	0.6	***	0.421	***	−0.119	0.196	−0.192	0.0398
	CD2	0.597	***	0.436	***	−0.146	0.112	−0.225	0.0158
Naive T cell	CCR7	0.59	***	0.389	***	0.163	0.0748	0.148	0.114
	LEF1	0.202	***	0.095	0.0775	−0.067	0.466	−0.137	0.145
	TCF7	0.147	*	−0.161	*	−0.132	0.15	−0.211	0.0237
	SELL	0.484	***	0.315	***	0.18	0.0497	0.137	0.142
Effector T cell	CX3CR1	0.406	***	0.318	***	−0.026	0.782	0.01	0.918
	FGFBP2	0.138	*	0.092	0.0896	−0.13	0.157	−0.194	0.0377
	FCGR3A	0.43	***	0.267	***	0.083	0.369	0.112	0.235
Effector memory T cell	PDCD1	0.416	***	0.257	***	−0.055	0.55	−0.134	0.154
	DUSP4	0.439	***	0.261	***	0.284	*	0.278	*
	GZMK	0.556	***	0.374	***	0.426	*	0.439	***
	GZMA	0.503	***	0.34	***	0.383	***	0.399	***
	IFNG	0.296	***	0.158	*	0.157	0.0865	0.16	0.0872
Resident memory T cell	CD69	0.587	***	0.413	***	0.307	**	0.274	*
	ITGAE	0.188	**	0.144	*	−0.034	0.708	−0.108	0.251
	CXCR6	0.564	***	0.394	***	0.229	0.0122	0.246	*
	MYADM	0.335	***	0.25	***	−0.102	0.268	−0.079	*
B cell	CD19	0.408	***	0.268	***	0.322	**	0.301	*
	CD79A	0.526	***	0.335	***	−0.023	0.804	−0.093	0.322
Monocyte	CD86	0.594	***	0.414	***	0.118	0.198	0.118	0.208
	CD115 (CSF1R)	0.587	***	0.391	***	0.186	0.0422	0.215	0.0209
TAM	CCL2	0.559	***	0.36	***	0.098	0.286	0.099	0.291
	CD68	0.405	***	0.213	***	0.183	0.0454	0.172	0.0667
	IL10	0.527	***	0.342	***	0.296	*	0.273	*
M1 Macrophage	INOS (NOS2)	0.117	0.025	0.07	0.198	−0.256	*	−0.231	0.013
	IRF5	0.142	*	0.124	0.022	0.455	***	0.467	***
	COX2 (PTGS2)	0.605	***	0.434	***	0.115	0.209	0.14	0.137
M2 Macrophage	CD163	0.514	***	0.319	***	0.339	***	0.328	***

(continued)

Table 2. *(continued)*

Description	Gene markers	LIHC				THYM			
		None		Purity		None		Purity	
		Cor	*p*	Cor	*p*	Cor	*p*	Cor	*p*
	VSIG4	0.55	***	0.376	***	0.207	0.0238	0.227	0.0148
	MS4A4A	0.543	***	0.34	***	0.269	*	0.251	*
Neutrophil	CD66b (CEACAM8)	0.116	0.025	0.084	0.12	0.053	0.566	0.029	0.756
	CD11b (ITGAM)	0.362	***	0.2	**	0.055	0.553	0.05	0.595
	CCR7	0.59	***	0.389	***	0.163	0.0748	0.148	0.114
Natural killer cell	KIR2DL1	0.096	0.064	0.056	0.3	0.104	0.259	0.135	0.149
	KIR2DL3	0.139	*	0.145	0.405	−0.026	0.781	−0.026	0.785
	KIR2DL4	0.217	***	0.147	*	0.184	0.0437	0.231	0.0132
	KIR3DL1	0.118	0.023	0.044	0.414	0.112	0.222	0.149	0.111
	KIR3DL2	0.221	***	0.132	0.014	0.112	0.224	0.12	0.203
	KIR3DL3	0.089	0.088	0.076	0.158	0.022	0.811	0.057	0.548
	KIR2DS4	0.154	*	0.178	**	0.043	0.642	0.079	0.399
Dendritic cell	HLA-DPB1	0.558	***	0.37	***	0.21	0.0216	0.203	0.0297
	HLA-DQB1	0.473	***	0.29	***	0.154	0.0924	0.115	0.22
	HLA-DRA	0.538	***	0.345	***	0.303	**	0.305	**
	HLA-DPA1	0.577	***	0.399	***	0.277	*	0.29	*
	BDCA-1 (CD1C)	0.568	***	0.421	***	−0.149	0.105	−0.219	0.0185
	BDCA-4 (NRP1)	0.316	***	0.24	***	0.123	0.181	0.187	0.0456
	CD11c (ITGAX)	0.593	***	0.438	***	0.305	**	0.315	**
Th1	TBX21 (T-bet)	0.529	***	0368	***	0.242	*	0.224	0.0159
	STAT4	0.479	***	0.39	***	0.078	0.395	0.09	0.336
	STAT1	0.276	***	0.152	*	0.259	*	0.292	*
	IFNG (IFN-g)	0.296	***	0.158	*	0.157	0.0865	0.16	0.0872
	TNF-a (TNF)	0.513	***	0.343	***	0.271	*	0.263	*
Th2	GATA3	0.601	***	0.45	***	−0.145	0.114	−0.225	0.0156
	STAT6	0.035	0.502	0.012	0.82	0.076	0.411	0.139	0.137
	STAT5A	0.357	***	0.203	**	−0.31	**	−0.291	*
	IL13	0.125	0.016	0.11	0.041	0.255	*	0.284	*
Tfh	BCL6	0.019	0.712	0.012	0.831	0.045	0.622	0.035	0.711
	IL21	0.079	0.127	0.005	0.922	−0.084	0.362	−0.096	0.31
Th17	STAT3	0.185	**	0.023	0.665	0.097	0.294	0.155	0.0977
	IL17A	0.103	0.048	0.112	0.037	0.223	0.0146	0.226	0.0152
Treg	FOXP3	0.299	***	0.193	**	0.215	0.0187	0.241	*
	CCR8	0.463	***	0.348	***	0.08	0.387	0.051	0.59
	STAT5B		0.105	−0.013	0.816	0.022	0.808	0.034	0.714
	TGFB1 (TGFb)	0.491	***	0.332	***	0.033	0.722	0.022	0.816
T cell exhaustion	PDCD1 (PD-1)	0.416	***	0.257	***	−0.055	0.55	−0.134	0.154
	CTLA4	0.45	***	0.296	***	0.248	*	0.245	*
	LAG3	0.331	***	0.25	***	0.138	0.131	0.138	0.141
	HAVCR2 (TIM-3)	0.575	***	0.386	***	0.315	**	0.306	**
	GZMB	0.32	***	0.156	*	0.133	0.147	0.115	0.219

Cor, R value of Spearman's correlation. None, correlation without adjustment. Purity, correlation adjusted by purity. *p<0.01; **p<0.001; ***p<0.0001. LIHC, liver hepatocellular carcinoma; TAM, tumor-associated macrophages; THYM, Thymoma.

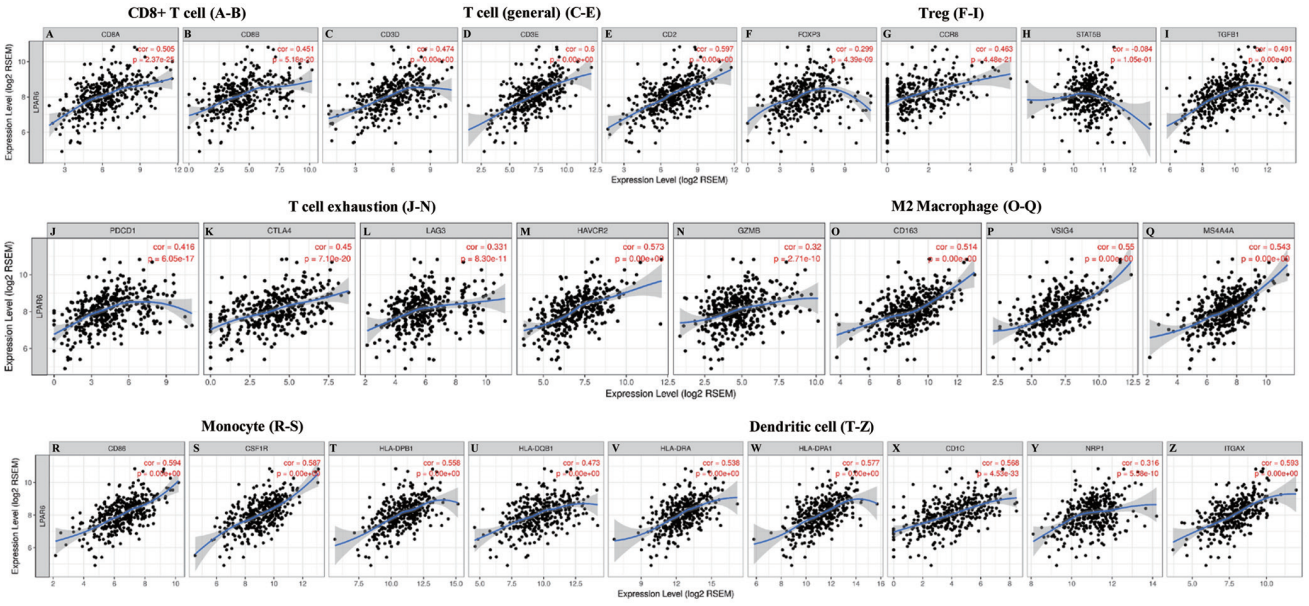

Fig. 6. Correlation analysis between *LPAR6* expression and immune marker sets in HCC. Markers include CD8A and CD8B of CD8+ T cells; CD3D, CD3E and CD2 of general T cells; FOXP3, CCR8, STAT5B and TGFB1 of Tregs; PDCD1, CTLA4, LAG3, HAVCR2 and GZMB of exhausted T cells; CD163, VSIG4 and MS4A4A of M2 macrophages; CD86 and CSF1R of monocytes; HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, CD1C, NRP1 and ITGAX of dendritic cells. (A–Z) Scatterplots of correlations between LPAR6 expression and gene markers of CD8+ T cells (A, B), general T cells (C–E), Tregs (F–I), T cell exhaustion (J–N), M2 macrophages (O–Q), monocytes (R–S) and dendritic cells (T–Z) in HCC. HCC, hepatocellular carcinoma; Tregs, T regulatory cells.

cates that LPAR6 may regulate dendritic cell infiltration in the tumor microenvironment of the LIHC patient and LPAR6 may be a novel target for HCC therapy.

Discussion

LPA receptors are GPCRs that bind the LPA and activate multiple cellular responses, such as cell proliferation, apoptosis, cytoskeletal rearrangements and motility.[34–36] To date, five LPA receptors (*LPAR*1-5) have been well characterized and extensively studied.[37] LPAR6 is a newly identified receptor, known as ARWH1, HYPT8, LAH3 and P2RY5, and the originally-referred-to purinergic receptor P2Y5 that is involved in inherited hair loss.[14,38] Although LPAR6 has not been extensively studied, it was reported that LPAR6 negatively regulates tumor cell migration in colorectal cancer,[15] and LPAR6 expression was down-regulated in P53-mutated cases. It was also reported that the LPA axis plays an important role in HCC by stimulating the recruitment and trans-differentiation of peritumoral fibroblasts into carcinoma-associated fibroblasts.[39,40] This gives us a clue that LPAR6 is involved in the tumor microenvironment. Nowadays, immunotherapy is applied as a novel treatment for

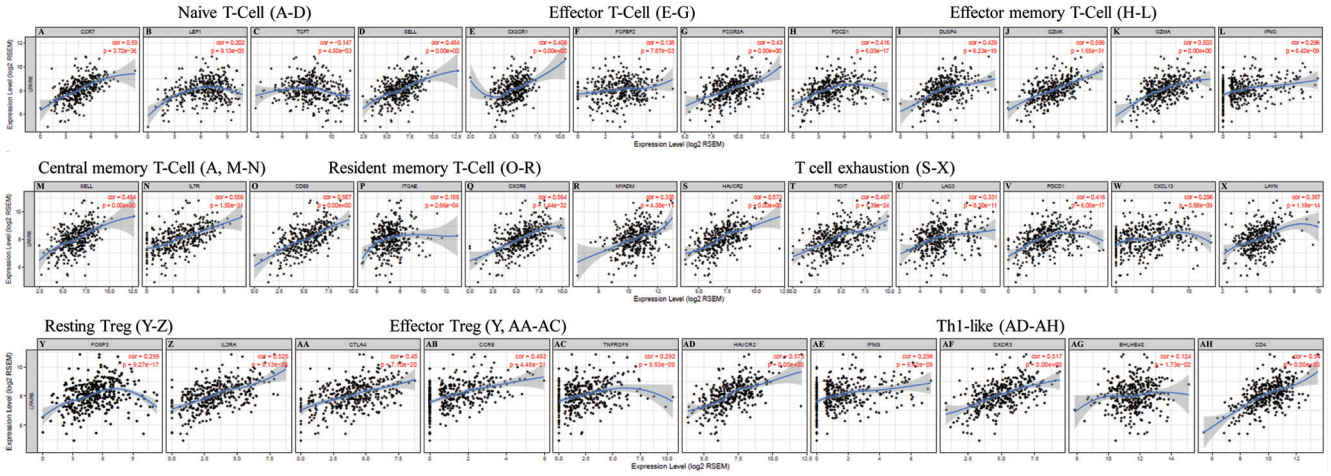

Fig. 7. Correlation analysis between *LPAR6* expression and various T cell marker sets in HCC. Scatterplots of correlations between LPAR6 expression and gene markers of naive T cells (CCR7, LEF1, TCF7, SELL) (A–D), effector T cells (CX3CR1, FGFBP2, FCGR3A) (E–G), effector memory T cells (PDCD1, DUSP4, GZMK, GZMA, IFNG) (H–L), central memory T cells (CCR7, SELL, IL7R) (A, M–N), resident memory T cells (CD69, ITGAE, CXCR6, MYADM) (O–R), T cell exhaustion (HAVCR2, TIGIT, LAG3, PDCD1, CXCL13, LAYN) (S–X), resting Tregs (FOXP3, IL2RA) (Y–Z), effector Tregs (FOXP3, CTLA4, CCR8, TNFRSF9) (Y, AA–AC), and Th1-like cells (HAVCR2, IFNG, CXCR3, BHLHE40, CD4) (AD–AH). HCC, hepatocellular carcinoma.

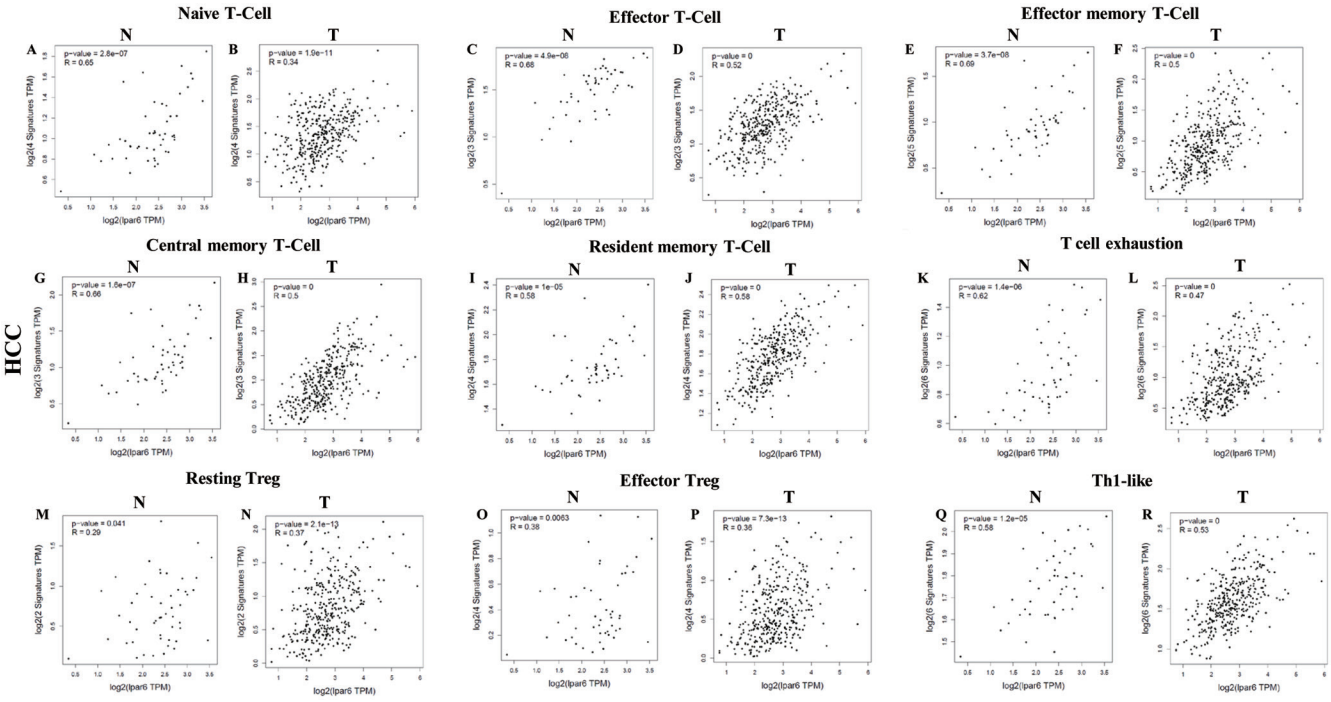

Fig. 8. Correlation analysis between *LPAR6* expression and various immune cells in normal and tumor tissue of HCC. (A–R) Scatterplots of correlations between LPAR6 expression and naive T cell (A, B), effector T cell (C, D), effector memory T cell (E, F), central memory T cell (G, H), resident memory T cell (I, J), T cell exhaustion (K, L), resting Treg (M, N), effector Treg (O, P), Th1-like (Q, R) in the normal and tissue of LIHC. HCC, hepatocellular carcinoma; LIHC, liver hepatocellular carcinoma.

patients with advanced cancer. Immunotherapy has shown good results in the treatment of NSCLC, but in HCC treatment, tumor immunotherapy is not effective.

In this study, we demonstrated that variations in LPAR6 expression levels are associated with the prognosis of different types of cancer. Higher expression level of LPAR6 is associated with a better prognosis of three types of cancers, including liver, breast and lung cancer. Here, we used the independent data set in Oncomine and 33 types of TCGA data in GEPIA2 to determine the mRNA expression level of LPAR6 in different types of cancer and the prognosis of the system. Differential expression patterns of LPAR6 between cancer and normal tissues is observed in many types of cancer.

When we looked into the Oncomine database, we found that the discrepancies in levels of LPAR6 expression in different cancer types among different databases might reflect the data collection approaches and underlying mechanisms pertinent to different biological properties. Nevertheless, in these databases we also found consistent prognostic correlation patterns between LPAR6 mRNA expression level in breast, bladder, colorectal, cervical, lung, esophageal and prostate cancers. The analysis of the TCGA database revealed that higher mRNA expression level of LPAR6 is associated with better prognosis in LGG, ACC, SKCM (Supplementary Fig. 2). Furthermore, analysis of data from two databases (Kaplan-Meier Plotter and PrognoScan) showed a higher mRNA level of LPAR6 expression was correlated with better prognosis in lung, breast, colorectal, bladder, ovarian and eye cancers (Fig. 2).

When we looked into two datasets in the PrognoScan database, we found that the mRNA expression level of LPAR6 could act as independent risk factors for prognosis in liver cancer and LUAD. That is, higher level of LPAR6 expression was shown to be associated with better prognosis of liver cancer in the early stages (stage 1 and stage 1+2) with the lowest HR for a better OS when LPAR6 was highly expressed. Considering these findings collectively, we believe that LPAR6 is a prognostic biomarker in HCC.

Another significant findings of this study is that the expression level of LPAR6 is correlated with a variety of immune infiltration levels in cancer (especially LIHC). Our results indicate that there is a strong positive correlation between the infiltration levels of T cells (CD8 + T and CD4 + T), neutrophils, macrophages, and dendritic cells and the mRNA expression level of LPAR6 in LIHC (Fig. 5). The correlation between LPAR6 expression and immune cell marker genes suggests a role for LPAR6 in regulating tumor immunology in these types of cancers. The possible explanation for this striking effect is that LPAR6 could orchestrate the functions of multiple immune marker genes. This supports the idea that LPAR6 tumor levels are important contributors to human disease and indicators of the prognosis of specific cancer types.

First, gene markers of M1 macrophages, such as PTGS2 and IRF5, show significant correlations with LPAR6 expression in LIHC (Table 2). Since macrophages are functionally plastic cells, M1 macrophages produce type 1 cytokines to prevent tumors from developing, whereas M2 macrophages induce type 2 cytokines to facilitate tumor growth. Especially in tumor tissue of LIHC, both NOS2 and IRF5 show significant correlations with LPAR6 expression and PTGS2 shows a significant correlation with LPAR6 expression in the tumor tissue of LIHC (Supplementary Table 3). These results reveal the potential regulating role of LPAR6 in depolarization of macrophages against tumor tissue that activated macrophages can be repolarized towards the opposite functional phenotypes by microenvironmental modifications and then inhibit tumor growth.

Second, our results indicated that LPAR6 has the potential to activate CD8+ T cells, naive T cells, effector T cells and natural killer cells and to inactivate Tregs and decrease T

cell exhaustion. CD8A, a crucial surface protein on T cells, is highly correlated with LPAR6 expression in LIHC, which are types of cancers with better prognosis. Moreover, CD8A negatively correlated in THYM, which has poor prognosis (Table 2). This pattern also occurs with the general T cell markers, such as CD3D, CD3E and CD2, and most markers of naive T cells, effector T cells, effector memory T cells and natural killer cells. Consider, LEF1, which has been proven as a predictor of better treatment response in acute myelocytic leukemia (AML), due to high expression level being associated with favorable RFS in patients and predicted a significantly better overall survival for AML patients.[41] Furthermore, the LPAR6 expression does not positively correlate with the Treg markers, such as STAT5B in LIHC (Table 2).

Third, different correlation patterns can be found between LPAR6 expression and the regulation of several markers of Th cells (Th1, Th2, follicular helper T cells, and Th17) in these different cancers. Interferon-gamma is a Th1 cytokine with both pro- and anti-cancer properties[42] and is highly correlated with LPAR6 expression in LIHC, whereas it did not demonstrate significant correlations in THYM (Table 2). Interleukin (IL)-13 is an important immunoregulatory cytokine which is mainly produced by activated Th2 cells, and is widely involved in tumorigenesis and development, fibrosis and inflammation.[43,44] We found that IL-13 is highly correlated with LPAR6 expression in THYM, but it did not demonstrate significant correlations in THYM (adjusted by purity) and a similar situation was observed for IL-21. So, these could be explanations as to why LPAR6 indicates a poor prognosis in THYM and a better prognosis in LIHC.

All these correlations listed above could be indications of a potential mechanism whereby LPAR6 regulates T cell functions in LIHC. Together, these findings suggest that the LPAR6 plays an important role in recruitment and regulation of effective T cells infiltrating in LIHC, leading to a better prognosis.

Conclusions

In this study, we provided possible mechanisms that explain why LPAR6 expression correlates with immune infiltration and better prognosis in some cancer types, especially in LIHC. Therefore, interactions between LPAR6 and the immune cells in the tumor microenvironment could represent a potential mechanism for the correlation of LPAR6 expression with immune infiltration and better prognosis in liver cancer and lung adenocarcinoma patients.

The results further confirm that LPAR6 is specifically correlated with immune infiltrating cells in LIHC, which suggests that LPAR6 plays a vital role in immune cell recruitment in HCC. LPAR6 and its regulation of tumor microenvironment may serve as a novel therapeutic target for HCC.

Thus, our study provides insights into understanding the potential role of LPAR6 in tumor immunology and its use as a cancer biomarker and novel therapy target for HCC.

Funding

This study was supported by grants from the National Natural Science Foundation (81630086, 82030099), the National Key R&D Program of China (2018YFC2000700), Shanghai Public Health System Construction Three-Year Action Plan (GWV-10.1-XK15), Innovative research team of high-level local universities in Shanghai for H.W. and the National Science Foundation of China (NSFC; 81702730) and the Start-up Plan for New Young Teacher of SHSMU (KJ30214190026) of JH.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Analyzed and interpreted the data (JH), wrote the manuscript (JH, MM), revised the manuscript for important intellectual content (HW).

Ethical approval

This project was permitted by Independent Ethics Committee of Shanghai Jiao Tong University School of Medicine.

Data Sharing statement

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

[1] Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet 2018;391(10127):1301–1314. doi:10.1016/S0140-6736(18)30010-2.
[2] Vafadar S. Immunotherapy for non-small cell lung cancer. JAAPA 2019;32(9):37–42. doi:10.1097/01.JAA.0000569792.99069.e6.
[3] Neeve SC, Robinson BW, Fear VS. The role and therapeutic implications of T cells in cancer of the lung. Clin Transl Immunology 2019;8(8):e1076. doi:10.1002/cti2.1076.
[4] Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, *et al*. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372(21):2018–2028. doi:10.1056/NEJMoa1501824.
[5] Cheng H, Sun G, Chen H, Li Y, Han Z, Li Y, *et al*. Trends in the treatment of advanced hepatocellular carcinoma: immune checkpoint blockade immunotherapy and related combination therapies. Am J Cancer Res 2019;9(8):1536–1545.
[6] Ravelli A, Roviello G, Cretella D, Cavazzoni A, Biondi A, Cappelletti MR, *et al*. Tumor-infiltrating lymphocytes and breast cancer: Beyond the prognostic and predictive utility. Tumour Biol 2017;39(4):1010428317695023. doi:10.1177/1010428317695023.
[7] Choi J, Gyamfi J, Jang H, Koo JS. The role of tumor associated macrophage in breast cancer biology. Histol Histopathol 2018;33(2):133–145. doi:10.14670/HH-11-916.
[8] Benevides L, da Fonseca DM, Donate PB, Tiezzi DG, De Carvalho DD, de Andrade JM, *et al*. IL17 promotes mammary tumor progression by changing the behavior of tumor cells and eliciting tumorigenic neutrophils recruitment. Cancer Res 2015;75(18):3788–3799. doi:10.1158/0008-5472.CAN-15-0054.
[9] Waniczek D, Lorenc Z, Snietura M, Wesecki M, Kopec A, Muc-Wierzgon M. Tumor associated macrophages and regulatory T cells infiltration and the clinical outcome in colorectal cancer. Arch Immunol Ther Exp 2017;65:445–454. doi:10.1007/s00005-017-0463-9.
[10] Seehawer M, Heinzmann F, D'Artista L, Harbig J, Roux PF, Hoenicke L, *et al*. Necroptosis microenvironment directs lineage commitment in liver cancer. Nature 2018;562(7725):69–75. doi:10.1038/s41586-018-0519-y.
[11] Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, *et al*. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. Cell 2017;169(7):1342–1356.e16. doi:10.1016/j.cell.2017.05.035.
[12] Taniguchi R, Inoue A, Sayama M, Uwamizu A, Yamashita K, Hirata K, *et al*. Structural insights into ligand recognition by the lysophosphatidic acid receptor LPA6. Nature 2017;548(7667):356–360. doi:10.1038/nature23448.
[13] Shimomura Y, Wajid M, Ishii Y, Shapiro L, Petukhova L, Gordon D, *et al*. Disruption of P2RY5, an orphan G protein-coupled receptor, underlies autosomal recessive woolly hair. Nat Genet 2008;40(3):335–339. doi:10.1038/ng.100.
[14] Takahashi K, Fukushima K, Onishi Y, Inui K, Node Y, Fukushima N, *et al*. Lysophosphatidic acid (LPA) signaling via LPA4 and LPA6 negatively regulates cell motile activities of colon cancer cells. Biochem Biophys Res Comm 2017;483(1):652–657. doi:10.1016/j.bbrc.2016.12.088.
[15] Sokolov E, Eheim AL, Ahrens WA, Walling TL, Swet JH, McMillan MT, *et al*. Lysophosphatidic acid receptor expression and function in human hepatocellular carcinoma. J Surg Res 2013;180(1):104–113. doi:10.1016/j.jss.2012.10.054.
[16] Ketscher A, Jilg CA, Willmann D, Hummel B, Imhof A, Rüsseler V, *et al*. LSD1 controls metastasis of androgen-independent prostate cancer cells through

PXN and LPAR6. Oncogenesis 2014;3:e120. doi:10.1038/oncsis.2014.34.
[17] Ishii S, Hirane M, Fukushima K, Tomimatsu A, Fukushima N, Tsujiuchi T. Diverse effects of LPA4, LPA5 and LPA6 on the activation of tumor progression in pancreatic cancer cells. Biochem Biophys Res Comm 2015;461(1):59–64. doi:10.1016/j.bbrc.2015.03.169.
[18] Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, *et al*. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia 2007;9(2):166–180. doi:10.1593/neo.07112.
[19] Jian He, Hui Wang. HspA1B is a prognostic biomarker and correlated with immune infiltrates in different subtypes of breast cancers. BioRxiv 2019: 725861. doi:10.1101/725861.
[20] Mizuno H, Kitada K, Nakai K, Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. BMC Med Genomics 2009;2:18. doi:10.1186/1755-8794-2-18.
[21] Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019;47(W1):W556–560. doi:10.1093/nar/gkz430.
[22] Lanczky A, Nagy A, Bottai G, Munkacsy G, Szabo A, Santarpia L, *et al*. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. Breast Cancer Res Treat 2016;160(3):439–446. doi:10.1007/s10549-016-4013-7.
[23] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Rodriguez IP, Chakravarthi BVSK, *et al*. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017;19(8):649–658. doi:10.1016/j.neo.2017.05.002.
[24] Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, *et al*. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010;38: W214–220. doi:10.1093/nar/gkq537.
[25] Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: Analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res 2018;46:D956–963. doi:10.1093/nar/gkx1090.
[26] Wang J, Vasaikar S, Shi Z, Zhang B, Greer M. WebGestalt: a more comprehensive, powerful, flexible and interactive gene set enrichment analysis toolkit. Nucleic Acids Res 2017;45:W130–137. doi:10.1093/nar/gkx356.
[27] Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, *et al*. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 2017;77:e108–110. doi:10.1158/0008-5472.CAN-17-0307.
[28] Danaher P, Warren S, Dennis L, D'Amico L, White A, Disis ML, *et al*. Gene expression markers of tumor infiltrating leukocytes. J Immunother Cancer 2017;5:18. doi:10.1186/s40425-017-0215-8.
[29] Duseja A. Staging of hepatocellular carcinoma. J Clin Exp Hepatol 2014; 4(Suppl 3):S74–79. doi:10.1016/j.jceh.2014.03.045.
[30] Ohtani H. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. Cancer Immun 2007;7:4.
[31] Azimi F, Scolyer RA, Rumcheva P, MoncrieffM, Murali R, McCarthy SW, *et al*. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. J Clin Oncol 2012;30:2678–2683. doi:10.1200/JCO.2011.37.8539.
[32] Facciabene A, Motz GT, Coukos G. T-regulatory cells: key players in tumor immune escape and angiogenesis. Cancer Res 2012;72:2162–2171. doi:10.1158/0008-5472.CAN-11-3687.
[33] Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer 2003;3:582–591. doi:10.1038/nrc1143.
[34] van Corven EJ, Groenink A, Jalink K, Eichholtz T, Moolenaar WH. Lysophosphatidate- induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell 1989;59:45–54. doi:10.1016/0092-8674(89)90868-4.
[35] Moolenaar WH, van Meeteren LA, Giepmans BN. The ins and outs of lysophosphatidic acid signaling. Bioessays 2004;26:870–881. doi:10.1002/bies.20081.
[36] Choi JW, Herr DR, Noguchi K, Yung YC, Lee CW, Mutoh T, *et al*. LPA receptors: subtypes and biological actions. Annu Rev Pharmacol Toxicol 2010;50:157–186. doi:10.1146/annurev.pharmtox.010909.105753.
[37] Pasternack SM, von Kugelgen I, Al Aboud K, Lee YA, Ruschendorf F, Voss K, *et al*. G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth. Nat Genet 2008;40:329–334. doi:10.1038/ng.84.
[38] Mazzocca A, Dituri F, De Santis F, Filannino A, Lopane C, Betz RC, *et al*. Lysophosphatidic acid receptor LPAR6 supports the tumorigenicity of hepatocellular carcinoma. Cancer Res 2015;75(3):532–543. doi:10.1158/0008-5472.CAN-14-1607.
[39] Mazzocca A, Dituri F, Lupo L, Quaranta M, Antonaci S, Giannelli G. Tumorsecreted lysophostatidic acid accelerates hepatocellular carcinoma progression by promoting differentiation of peritumoral fibroblasts in myofibroblasts. Hepatology 2011;54:920–930. doi:10.1002/hep.24485.
[40] Fu Y, Zhu H, Wu W, Xu J, Chen T, Xu B, *et al*. Clinical significance of lymphoid enhancer- binding factor 1 expression in acute myeloid leukemia. Leuk Lymphoma 2014;55(2):371–377. doi:10.3109/10428194.2013.805759.
[41] Ganapathi SK, Beggs AD, Hodgson SV, Kumar D. Expression and DNA methylation of TNF, IFNG and FOXP3 in colorectal cancer and their prognostic significance. Br J Cancer 2014;111(8):1581–1589. doi:10.1038/bjc.2014.477.
[42] Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A, Puri RK. IL-13 signaling through the IL-13 alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med 2006;1(1):99–106. doi:10.1038/nm1332.
[43] Shimamura T, Fujisawa T, Husain SR, Joshi B, Puri RK. Interleukin 13 mediates signal transduction through interleukin 13 receptor alpha2 in pancreatic ductal adenocarcinoma: role of IL-13 Pseudomonas exotoxin in pancreatic cancer therapy. Clin Cancer Res 2010;2(2):577–586. doi:10.1158/1078-0432.CCR-09-2015.