LPAR2 correlated with different prognosis and immune cell infiltration in head and neck squamous cell carcinoma and kidney renal clear cell carcinoma

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Abstract

Background: Lysophosphatidic acid (LPA) and its receptors play a key role in regulating cancer progression. Upregulation of LPA receptor 2 (LPAR2) plays a role in carcinogenesis; however, the exact role of LPAR2 in tumors remains elusive. This study aims to explore the correlation between LPAR2 expression with tumor prognosis and immune infiltration in pan-cancers.

Materials and methods: The expression of LPAR2 in pan-cancers was analyzed using the Online Cancer Microarray Database (Oncomine), Tumor Immune Estimation Resource (TIMER), and UALCAN databases. The effects of LPAR2 on the clinical prognosis in pan-cancer were examined using the Kaplan–Meier plotter (KM plotter) as well as Gene Expression Profiling Interactive Analysis (GEPIA), UALCAN, and Human Protein Atlas (HPA) databases. Moreover, the R software program was applied for validation of expression and prognostic value of LPAR2 in tumor patients in the Cancer Genome Atlas (TCGA) dataset and the Gene Expression Omnibus (GEO) database. The relationship between the expression level of LPAR2 and the clinical and molecular criteria of head and neck squamous cell carcinoma (HNSC) and kidney renal clear cell carcinoma (KIRC) was analyzed using UALCAN, whereas the relationship between LPAR2 expression and prognosis in patients with HNSC and KIRC with different clinical characteristics was examined using the KM plotter. Furthermore, the correlation between LPAR2 expression and tumor immune infiltration was examined using TIMER. The correlation between LPAR2 expression and gene markers of tumor immune infiltrates was analyzed using TIMER and GEPIA. In addition, the cBioPortal for Cancer Genomics was used to calculate the mutations, methylations, and altered neighbor genes of LPAR2.

Results: The expression of LPAR2 was significantly correlated with the outcome of multiple types of cancer, especially HNSC and KIRC. Furthermore, high expression of LPAR2 was significantly associated with various immune markers in the immune cell subsets of HNSC and KIRC.

Conclusions: High expression of LPAR2 plays significantly different prognostic roles in HNSC and KIRC possibly owing to its association with different immune markers. LPAR2 is correlated with tumor immune cell infiltration and is a valuable prognostic biomarker for HNSC and KIRC. However, further experiments are required to validate these findings.
Introduction
Lysophosphatidic acid (LPA, 1-acyl-2-hemolytic-sn-glycerin-3-phosphate) is a bioactive glycerophosphatidic acid, which is a naturally occurring lysophospholipid and is abundantly found in the human body [1, 2]. Lipopolysaccharides, lysophosphatidylethanolamine, and lysophosphatidylcholine are hydrolyzed by autotaxin to produce LPA in plasma, serum, and adipocytes [3]. LPA serves as a growth factor by activating distinct high-affinity G protein-coupled receptors (GPCRs), which promote the growth, differentiation, migration, division, and survival of various cell types [4, 5]. LPA has several GPCRs known as LPA receptors (LPARs) [6]. According to their homology, LPARs can be divided into six types, namely, LPAR1, LPAR2, LPAR3, LPAR4, LPAR5, and LPAR6, which can be grouped into two subfamilies, namely, endothelial differentiation gene (EDG) family (LPAR1–3) and purinergic receptor family (LPAR4–6) [7]. LPARs contain seven transmembrane domains, three intracellular loops, and three extracellular loops [8]. The LPAR signaling pathway produces different results in different environments and cell types, and at least two Gα subunits are involved (Gαq/11, Gα12/13, Gαi/o, and GαS) that activate different downstream pathways [9, 10]. Several signaling pathways, such as RhoA, phospholipase C, PI3K/PAK1/ERK, Ras–Raf–MEK–ERK, and Rac pathways, are activated by Gαq/11, Gα12/13, Gαi/o, and GαS [9, 11]. Owing to the presence of similar G protein types, the six LPARs perform similar biological functions [12]. Multiple studies have revealed the key roles of LPA and LPARs in various cancer tissues, such as in breast, lung, liver, pancreatic, ovarian, and thyroid cancers and neuroblastoma [13, 14].

Although many studies have described the expression and function of LPAR1 and LPAR3 in several tumors, studies on LPAR2 are limited [15]. Several studies have reported that LPAR2 is aberrantly expressed in several tumors, including breast, colorectal, kidney, and pancreatic cancers [16–19], and promotes robust activation of RhoA to mediate cell migration [20]. A recent study demonstrated that LPAR2 regulated cell–cell adhesion of neural crest cells by internalizing N-cadherin downstream of LPAR2 [21]. In addition, a study reported that LPAR2 is significantly associated with LPA-induced expression of interleukin (IL)-6 and IL-8, which promoted breast cancer progression [22, 23]. However, the mechanism of action of LPAR2 in tumors appears diverse and remains unclear [24].

In this study, we systematically investigated the expression of LPAR2 and its relationship with pan-cancer prognosis using the Oncomine, TIMER, UALCAN, GEPIA, KM plotter and HPA databases, as well as expression and survival analysis of LPAR2 in the TCGA and GEO data was validated by R software. Furthermore, we examined the relationship between LPAR2 expression and the clinical and molecular criteria of HNSC and KIRC using UALCAN. Subsequently, we investigated the relationship between LPAR2 expression and the prognosis of patients with HNSC and KIRC with different clinical characteristics using the KM plotter. In addition, we analyzed the correlation between LPAR2 and tumor-infiltrating immune cells in the microenvironment of pan-cancer using TIMER and GEPIA. Lastly, we used the cBioPortal for Cancer Genomics online tool to analyze the alterations, mutations, methylations, and pathways of LPAR2. Therefore, in this study, we demonstrated a potential mechanism of action of LPAR2, examined the prognostic role of LPAR2 in HNSC and KIRC, and identified LPAR2 as a key factor in regulating the immune microenvironment of HNSC and KIRC. The overall design and workflow of this study is presented in Fig. 1.

Results
Assessment of LPAR2 expression in different cancers and normal tissues
On analyzing the mRNA expression levels of LPAR2 in pan-cancer and normal tissues using Oncomine, we found that LPAR2 expression was higher in bladder, brain and central nervous system (CNS), breast, colorectal, kidney, and lung cancers and lymphoma than in normal control tissues (Fig. 2A). However, LPAR2 expression was lower in kidney cancer, leukemia, lung cancer, lymphoma and sarcoma tissues than in normal control tissues (Fig. 2A). In addition, a Kaplan–Meier survival analysis of different cancers and normal tissues was performed using TIMER and UALCAN. Table 1 summarizes the detailed findings of specific tumor types. Furthermore, we assessed differences in LPAR2 expression in pan-cancer using the TIMER databases and found that LPAR2 expression was significantly higher in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), HNSC, KIRC, liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach
Fig. 1 Analysis workflow of this research
adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC) than in the adjacent normal tissues (Fig. 2B). However, LPAR2 expression was significantly lower in kidney chromophobe (KICH) and thyroid carcinoma (THCA) than in the adjacent normal tissues (Fig. 2B). Subsequently, we examined LPAR2 expression using UALCAN and found that the mRNA expression levels of LPAR2 were significantly higher in BLCA, BRCA, cervical squamous cell carcinoma and endocervical adenocarcinoma (CEC), glioblastoma multiforme (GBM), HNSC, KIRC, kidney renal papillary cell carcinoma (KIRp), LIHC, LUAD, LUSC, PRAD, READ, STAD, and UCEC than in normal control tissues (Figs. 2C, 3). However, LPAR2 expression was significantly lower in KICH and THCA than in normal control tissues (Fig. 3). Differences in LPAR2 expression between tumors and normal adjacent tissue samples are demonstrated in Fig. 1C. To validate these results, we used R software to analyze expression of LPAR2 in pan-cancers via the TCGA databases (Fig. 4A). As a result, we observed the same trend in the expression of LPAR2 in pan-cancers (Fig. 4A).

Relationship between LPAR2 expression and prognosis in patients with cancer
We used KM plotter to determine the correlation between LPAR2 expression and the survival of patients with pan-cancer and those with normal tissues (Figure S1). A significant correlation was observed between LPAR2 expression and prognosis in BLCA, BRCA, CESC, HNSC, KIRC, STAD, THYM, and UCEC (Fig. 4B–N). In addition, we found that high LPAR2 expression was significantly associated with a worse prognosis in patients with BRCA (overall survival [OS], HR = 1.42 [1.16–1.74], P = 0.00069; distant metastasis-free survival [DMFS], HR = 1.31 [1.12–1.53], P = 0.00083), STAD (OS, HR = 1.24 [1.04–1.49], P = 0.017; first progression [FP], HR = 1.26 [1.02–1.55], P = 0.028; and post-progression survival [PPS], HR = 1.33 [1.06–1.67], P = 0.014) and KIRC (OS, HR = 2.44 [1.8–3.31], P = 3.5e–09) (Fig. 4C, E, H–K). On the contrary, high LPAR2 expression was associated with improved prognosis in patients with BLCA (OS, HR = 0.68 [0.47–0.98], P = 0.036), CESC (OS, HR = 0.52 [0.32–0.86], P = 0.0089), HNSC (OS, HR = 0.65 [0.49–0.86], P = 0.0023), THYM (OS,
HR = 0.17 [0.04 – 0.68], *P* = 0.0046, UCEC (OS, HR = 0.59 [0.38 – 0.9], *P* = 0.014, RFS, HR = 0.54 [0.32 – 0.91], *P* = 0.018) and BRCA (RFS, HR = 0.8 [0.71 – 0.89], *P* = 7.3e − 05) (Fig. 4B, F, G, L, M, N). However, no significant correlation was observed between the mRNA expression levels of LPAR2 and the prognosis of other cancers (Figure S1). Furthermore, we assessed the relationship between LPAR2 expression and the prognosis of multiple cancers using GEPIA (Figure S2) and found that high mRNA expression of LPAR2 was associated with a worse prognosis in patients with KIRC (OS, HR = 2.1, *P* = 3.6e − 06; disease-free survival [DFS], HR = 1.9, *P* = 9e − 04), PRAD (OS, HR = 7.7, *P* = 0.024), and CHOL (DFS, HR = 2.6, *P* = 0.048) (Fig. 5A, C–E). Furthermore, high mRNA expression of LPAR2 was correlated with better OS in patients with HNSC (HR = 0.71, *P* = 0.012) and THYM (HR = 0.11, *P* = 0.013) (Fig. 5B, F). However, it was not significantly correlated with OS and DFS in patients with BRCA (OS, HR = 0.85, *P* = 0.49; DFS, HR = 0.74, *P* = 0.29) and other tumors (Figure S2). In UALCAN databases, we found that higher expression of LPAR2 was associated with improved prognosis in patients with ACC (*P* = 0.00055), as well as with worse prognosis with KIRC (*P* < 0.0001) (Fig. 5G, I). Upregulation of LPAR2 might be correlated with good prognosis in HNSC patients, but this correlation was not statistically significant (Fig. 5H). Nevertheless, in UACLAN, no significant correlation was observed between LPAR2 expression and the prognosis of other cancers (Figure S3).

Likewise, to validate these results, survival analysis of LPAR2 in pan-cancers of the TCGA databases was performed using the survival package via R software (Figure S4). Our results indicated that high expression level of LPAR2 was significantly associated with a worse OS in patients with ACC (HR = 2.35 [1.08 – 5.11], *P* = 0.031), KIRC (HR = 1.99 [1.46 – 2.71], *P* < 0.001) and MESO (OS, HR = 1.74 [1.08 – 2.81], *P* = 0.023) (Fig. 5 J, L, M).

Table 1: The significant changes of LPAR2 expression in cancers vs normal tissue in oncomine database

| Cancer            | Cancer type                                      | *P*-value | Fold change | Rank (%) | Sample | Reference            |
|-------------------|--------------------------------------------------|-----------|-------------|----------|--------|----------------------|
| Bladder Cancer    | Superficial Bladder Cancer vs. Normal            | 4.02E-23  | 5.967       | 1%       | 41     | Sanchez-Carbayo Bladder 2 |
|                   | Infiltrating Bladder Urothelial Carcinoma vs.    | 5.43E-11  | 2.342       | 3%       | 367    | Sanchez-Carbayo Bladder 2 |
|                   | Normal                                           |           |             |          |        |                      |
|                   | Superficial Bladder Cancer vs. Normal            | 1.35E-7   | 1.582       | 3%       | 375    | Dyrekjot Bladder 3    |
|                   | Superficial Bladder Carcinoma vs. Normal         | 8.31E-6   | 1.504       | 4%       | 652    | Lee Bladder           |
| Brain and CNS     | Anaplastic Astrocytoma vs. Normal                | 4.10E-5   | 2.255       | 8%       | 1521   | Sun Brain             |
| Cancer            | Mixed Lobular and Ductal Breast Carcinoma vs.    | 3.13E-9   | 1.889       | 1%       | 50     | TCGA Breast           |
|                   | Normal                                           |           |             |          |        |                      |
|                   | Invasive Lobular Breast Carcinoma vs. Normal     | 1.24E-9   | 1.791       | 8%       | 1551   | TCGA Breast           |
|                   | Invasive Breast Carcinoma vs. Normal             | 1.07E-11  | 1.756       | 10%      | 1942   | TCGA Breast           |
|                   | Medullary Breast Carcinoma vs. Normal            | 1.26E-7   | 1.619       | 10%      | 1742   | Curtis Breast         |
| Colorectal Cancer | Rectal Adenoma vs. Normal                        | 1.09E-6   | 2.735       | 3%       | 549    | Sabates-Bellver Colon |
| Kidney Cancer     | Papillary Renal Cell Carcinoma vs. Normal        | 2.40E-13  | 1.532       | 2%       | 220    | Jones Renal           |
|                   | Chromophobe Renal Cell Carcinoma vs. Normal      | 4.03E-6   | 1.673       | 6%       | 663    | Jones Renal           |
|                   | Renal Oncocytoma vs. Normal                      | 5.97E-9   | 1.969       | 6%       | 650    | Jones Renal           |
|                   | Clear Cell Renal Cell Carcinoma vs. Normal       | 1.78E-10  | 1.688       | 7%       | 801    | Jones Renal           |
|                   | Renal Pelvis Urothelial Carcinoma vs. Normal     | 5.02E-6   | 1.870       | 8%       | 933    | Jones Renal           |
|                   | Clear Cell Renal Cell Carcinoma vs. Normal       | 4.38E-19  | -2.100      | 4%       | 437    | Jones Renal           |
|                   | Papillary Renal Cell Carcinoma vs. Normal        | 9.72E-13  | -1.613      | 4%       | 480    | Jones Renal           |
| Leukemia          | T-Cell Acute Lymphoblastic Leukemia vs. Normal   | 3.28E-9   | -8.139      | 2%       | 110    | Andersson Leukemia    |
|                   | Acute Myeloid Leukemia vs. Normal                | 1.17E-9   | -6.503      | 2%       | 181    | Andersson Leukemia    |
|                   | B-Cell Acute Lymphoblastic Leukemia vs. Normal   | 8.15E-8   | -9.264      | 9%       | 859    | Andersson Leukemia    |
| Lung cancer       | Lung Adenocarcinoma vs. Normal                   | 2.50E-14  | 1.623       | 4%       | 612    | Selamat Lung          |
|                   | Small Cell Lung Carcinoma vs. Normal             | 8.31E-5   | -4.067      | 9%       | 741    | Bhattacharjee Lung    |
| Lymphoma          | Diffuse Large B-Cell Lymphoma vs. Normal         | 2.31E-5   | 1.551       | 6%       | 1085   | Brune Lymphoma        |
|                   | Unspecified Peripheral T-Cell Lymphoma vs.       | 6.08E-12  | -1.797      | 3%       | 214    | Piccaluga Lymphoma    |
|                   | Normal                                           |           |             |          |        |                      |
| Other cancer      | Testicular Seminoma vs. Normal                   | 7.73E-8   | 1.859       | 3%       | 284    | Sperger Others        |
| Sarcoma           | Gastrointestinal Stromal Tumor vs. Normal        | 3.90E-10  | -4.256      | 2%       | 269    | Cho Gastric           |

HR = 0.17 [0.04 – 0.68], *P* = 0.0046, UCEC (OS, HR = 0.59 [0.38 – 0.9], *P* = 0.014, RFS, HR = 0.54 [0.32 – 0.91], *P* = 0.018) and BRCA (RFS, HR = 0.8 [0.71 – 0.89], *P* = 7.3e − 05) (Fig. 4B, F, G, L, M, N). However, no significant correlation was observed between the mRNA expression levels of LPAR2 and the prognosis of other cancers (Figure S1). Furthermore, we assessed the relationship between LPAR2 expression and the prognosis of multiple cancers using GEPIA (Figure S2) and found that high mRNA expression of LPAR2 was associated with a worse prognosis in patients with KIRC (OS, HR = 2.1, *P* = 3.6e − 06; disease-free survival [DFS], HR = 1.9, *P* = 9e − 04), PRAD (OS, HR = 7.7, *P* = 0.024), and CHOL (DFS, HR = 2.6, *P* = 0.048) (Fig. 5A, C–E). Furthermore, high mRNA expression of LPAR2 was correlated with better OS in patients with HNSC (HR = 0.71, *P* = 0.012) and THYM (HR = 0.11, *P* = 0.013) (Fig. 5B, F). However, it was not significantly correlated with OS and DFS in patients with BRCA (OS, HR = 0.85, *P* = 0.49; DFS, HR = 0.74, *P* = 0.29) and other tumors (Figure S2). In UALCAN databases, we found that higher expression of LPAR2 was associated with improved prognosis in patients with ACC (*P* = 0.00055), as well as with worse prognosis with KIRC (*P* < 0.0001) (Fig. 5G, I). Upregulation of LPAR2 might be correlated with good prognosis in HNSC patients, but this correlation was not statistically significant (Fig. 5H). Nevertheless, in UACLAN, no significant correlation was observed between LPAR2 expression and the prognosis of other cancers (Figure S3).
On the other hand, high LPAR2 expression was associated with improved prognosis in patients with HNSC (HR = 0.74 [0.56 – 0.96], \( P = 0.025 \)), OV (HR = 0.74 [0.57 – 0.96], \( P = 0.023 \)) and STAD (OS, HR = 0.70 [0.51 – 0.97], \( P = 0.035 \)) (Fig. 5 K, N, O).

Taken together, the combination of OS, RFS, DFS and DMFS, and concern of bias, our findings illustrated the expression levels and prognostic value of LPAR2 in several types of cancers, especially HNSC and KIRC, suggesting that high LPAR2 expression plays significantly
Fig. 4 (See legend on previous page.)
different prognostic roles in HNSC and KIRC. Thus, we performed LPAR2 expression analyses and survival analyses in HNSC and KIRC using GEO databases in the end. Results of differential expression analysis showed that LPAR2 expression was significantly higher in HNSC and KIRC than in normal control tissues in GSE30784, GSE31056, GSE53757 and GSE15641 (P < 0.01) (Fig. 6A–E). However, survival analysis of GSE686, GSE65858, GSE167573 and GSE22541 showed that no further significant correlations were found between LPAR2 expression and the prognosis of HNSC and KIRC (P > 0.05) (Figure S5A–D). These inconsistencies might be due to limited sample sizes of HNSC and KIRC in GEO databases and differences in data collection methods as well as biases in methods of adjustment. Therefore, much further experimental validation is needed to investigate the link between the expression of LPAR2 and prognosis in cancer patients with HNSC and KIRC as well as other kinds of cancers.

Relationship between protein expression of LPAR2 and prognosis in patients with HNSC and KIRC

After analyzing the mRNA expression of LPAR2 and its relationship with the prognosis of patients with HNSC and KIRC, we investigated the protein expression of LPAR2 and its correlation with the prognosis of patients with HNSC and KIRC using the HPA database. As demonstrated in Fig. 6 F–I, the protein expression of LPAR2 was moderate in HNSC and KIRC tissues and low in the corresponding normal tissues. Relevant clinical data was shown in Table S1. Furthermore, according to the data obtained from the HPA, the relationship between the protein expression of LPAR2 and prognosis was similar to that between the mRNA expression of LPAR2 and prognosis. Moreover, high protein expression of LPAR2 was associated with worse OS in patients with KIRC (P = 3.5e-9) but with improved OS in patients with HNSC (P = 0.0023) (Fig. 6 J–K). The related clinical data were exhibited in Table S2 and Table S3.

Relationship between mRNA expression of LPAR2 and clinical characteristics of patients with HNSC and KIRC

Given that LPAR2 expression plays significantly different prognostic roles in HNSC and KIRC, we used UALCAN and TCGA to examine the relationship between LPAR2 expression and the clinical characteristics of patients with HNSC and KIRC. For the criterion of tumor stage, we found that LPAR2 expression was significantly higher in patients with stage 1–4 HNSC than in patients in the control group (P < 0.001) (Fig. 7D). For the criterion of race, the mRNA expression of LPAR2 was higher in the Caucasian and African–American patients with HNSC than in patients in the control group (P < 0.001); however, there was no significant difference in LPAR2 expression between the Asian patients with HNSC and those in the control group (P > 0.05) (Fig. 7C). In addition, LPAR2 expression was upregulated in both men and women with HNSC (P < 0.001) (Fig. 7B) in the age groups of 21–40 years (P < 0.001), 41–60 years (P < 0.001), 61–80 years, and 81–100 years (P < 0.001) (Fig. 7A). These findings suggested that the mRNA expression of LPAR2 was significantly higher in patients with HNSC than in patients in the control group (P < 0.01 and P < 0.001, respectively), irrespective of tumor grade, HPV expression status, nodal metastasis status, and mutation status (Fig. 7E, F, G, H).

In patients with KIRC, LPAR2 expression was upregulated in patients with tumor stages 3 and 4 (P < 0.001) (Fig. 8D). However, there was no significant difference in LPAR2 expression between patients with tumor stages 1–2 and those in the control group (P > 0.05) (Fig. 8D). Similar to HNSC, LPAR2 expression was significantly higher in the Caucasian and African–American patients with KIRC than in patients in the control group (P < 0.001); whereas there was no significant difference in LPAR2 expression between the Asian patients with KIRC and those in the control group (P > 0.05) (Fig. 8C). In addition, LPAR2 expression was upregulated in both men and women with KIRC (P < 0.001) (Fig. 8B). Meanwhile, we found that LPAR2 expression was upregulated in patients with KIRC in the age groups of 21–40 years (P < 0.05), 41–60 years (P < 0.01), and 61–80 years (P < 0.001) but not in the age group of 81–100 years (P > 0.05) (Fig. 8A). Our findings also suggested that the mRNA expression of LPAR2 was higher in patients with grade 3–4 KIRC than in patients in the control group (P < 0.001); nonetheless there was no significant difference between the mRNA expression of LPAR2 in patients with grade 1–2 KIRC and those in the control group (P > 0.05) (Fig. 8E). Furthermore, the mRNA expression of LPAR2 was higher in patients with node-positive KIRC than in patients with node-negative KIRC; however, it was higher in both conditions.
Fig. 5 (See legend on previous page.)
node-positive and node-negative patients than in patients in the control group \((P < 0.01 \text{ and } P < 0.001\), respectively) (Fig. 8F). These findings suggested that \(\text{LPAR2}\) expression was associated with tumor stage, tumor grade, and lymph node metastasis in patients with KIRC, and with race in patients with HNSC and KIRC.

**Relationship between mRNA expression of \(\text{LPAR2}\) and prognosis in patients with HNSC and KIRC with different clinical characteristics**

For a better understanding of the mechanisms of \(\text{LPAR2}\) expression in HNSC and KIRC, we assessed the relationship between mRNA expression of \(\text{LPAR2}\) and prognosis in patients with HNSC and KIRC with different clinical characteristics in KM plotter. Higher mRNA expression of \(\text{LPAR2}\) was associated with better OS in HNSC tumor stage 2–4 (stage 2, HR = 0.45 [0.2–0.99], \(P = 0.042\); stage 3, HR = 0.35 [0.14–0.88], \(P = 0.019\); stage 4, HR = 0.55 [0.38–0.79], \(P = 0.00094\)). However, no significant correlation was observed between the mRNA expression of \(\text{LPAR2}\) and OS in patients with HNSC tumor stage 1 \((P > 0.05)\) (Fig. 9A–D). \(\text{LPAR2}\) overexpression was correlated with better OS in men with HNSC (HR = 0.58 [0.42–0.81], \(P = 0.0012\); however, no significant correlation was found between \(\text{LPAR2}\) expression and OS in women with HNSC \((P = 0.5)\) (Fig. 9E–F). For the criterion of race, higher mRNA expression of \(\text{LPAR2}\) was correlated with better OS in the White patients (HR = 0.64 [0.47–0.86], \(P = 0.0032\)) but not in the Black/Asian patients \((P > 0.05)\)
Furthermore, we found that upregulated mRNA expression of \textit{LPAR2} was associated with improved OS in patients with HNSC grade 2 (HR = 0.67 [0.47–0.96], \(P = 0.029\)) and grade 3 (HR = 0.33 [0.19–0.57], \(P = 3.5e-05\)) (Fig. 8J–K) but not in grade 1 (\(P > 0.05\)) (Fig. 9I). For the criterion of mutation status, the results indicated that high mRNA expression level of \textit{LPAR2} were correlated with improved OS in the low-\textit{LPAR2}-mutation-burden group (HR = 0.46 [0.29–0.74], \(P = 0.00095\)) (Fig. 9M). However, in the high-\textit{LPAR2}-mutation-burden group, no significant relationship was observed between the mRNA expression of \textit{LPAR2} and prognosis (\(P > 0.05\)) (Fig. 9L).

In patients with KIRC, upregulated expression of \textit{LPAR2} was associated with worse OS in patients with tumor stage 1 (HR = 2.07 [1.08–3.97], \(P = 0.024\)), tumor stage 2 (HR = 2.42 [1.08–5.41], \(P = 0.026\)), and tumor stage 4 (HR = 1.85 [1.04–3.31], \(P = 0.034\)) (Fig. 10A, C, D) but not in tumor stage 2 (\(P > 0.05\)) (Fig. 10B). In addition, high mRNA expression of \textit{LPAR2} was associated with shorter OS in the White patients (HR = 2.55 [1.86–3.51], \(P = 2.1e-09\)) but not in the Black/Asian patients (\(P > 0.05\)) (Fig. 10I–J). \textit{LPAR2} overexpression was associated with worse OS in men (HR = 2.76 [1.88–4.04], \(P = 5.7e-08\)) and women (HR = 3.83 [2.7–7.36], \(P = 1.4e-05\)) with KIRC (Fig. 10G–H). In addition, high \textit{LPAR2} expression was associated with worse OS in patients with KIRC grade 2–4 (grade 2, HR = 2.94 [1.31–6.6], \(P = 0.0062\); grade 3, HR = 2.72 [1.46–5.05], \(P = 0.001\); and grade 4, HR = 1.75 [1.02–3.03], \(P = 0.041\)) (Fig. 10K–M) and in the high- and low-\textit{LPAR2}-mutation-burden groups (high, HR = 2.15 [1.23–3.74], \(P = 0.0058\); low, HR = 3.01 [1.33–6.83], \(P = 0.056\)) (Fig. 10N–O).

These results suggested that \textit{LPAR2} expression influenced the prognosis of patients with HNSC of high stage and grade. Upregulated expression of \textit{LPAR2} was beneficial to men with HNSC or patients with low \textit{LPAR2} mutation burden and was significantly associated with prognosis in White patients with HNSC and KIRC.

Association between \textit{LPAR2} expression and immune cell infiltration in HNSC and KIRC

Tumor-infiltrating lymphocytes are independent predictors of tumor stage, grade, and lymph node status in cancers [25, 26]. Therefore, we used the TIMER database to analyze the relationship between \textit{LPAR2} expression and the degree of immune cell infiltration in HNSC and KIRC (Fig. 11) and found that \textit{LPAR2} expression was significantly correlated with tumor purity (\(R = 0.2, P = 7.74e-06\)), B cell infiltration (\(R = 0.217, P = 1.70e-05\)), and CD4+ T cell infiltration (\(R = 0.149, P = 1.07e-03\)) but not with the infiltration of CD8+ T cells, macrophages, neutrophils, and DCs in patients with HNSC (Fig. 11A). In patients with KIRC, \textit{LPAR2} expression was significantly correlated with tumor purity (\(R = 0.155, P = 8.49e-04\)), B cell infiltration (\(R = 0.168, P = 2.94e-04\)), CD4+ T cell infiltration (\(R = 0.242, P = 1.46e-07\)), neutrophil
infiltration \((R = 0.197, P = 2.09 \times 10^{-5})\), and DC infiltration \((R = 0.141, P = 2.66 \times 10^{-3})\) (Fig. 11A) but not with the infiltration of CD8+ T cells and macrophages (Fig. 11A). We further analyzed the correlation between LPAR2 expressions and immune cell infiltration in patients with HNSC and KIRC by generating KM plots using the TIMER database. The results demonstrated that B-cell infiltration was significantly correlated with the prognosis of HNSC \((P = 0.045)\) (Fig. 11B), and a significant correlation was observed between the mRNA expression of LPAR2 and prognosis in patients with KIRC \((P < 0.001)\) (Fig. 11B). These results suggest that LPAR2 is important for regulating immune cell infiltration in HNSC and KIRC. Moreover, LPAR2 is more important for regulating tumor purity and the infiltration of B cells and CD4+ T cells.
cells in HNSC as well as the infiltration of neutrophils and DCs in KIRC.

Relationship between LPAR2 and immune marker expression

Given that LPAR2 is important for regulating immune cell infiltration in HNSC and KIRC, we assessed the relationship between LPAR2 expression and immune cell infiltration based on the immunological markers of HNSC and KIRC using the TIMER and GEPIA databases. In addition, we evaluated the relationship between LPAR2 expression and several immunological marker subsets, including total T cells, B cells, CD8+ T cells, tumor-associated macrophages (TAMs), monocytes, M1 and M2 macrophages, natural killer (NK) cells, neutrophils, DCs, T follicular helper (Tfh) cells, type 1 T helper

Fig. 10 the relationship between the LPAR2 mRNA expression and prognosis in KIRC patients with different clinical characteristics in Kaplan–Meier plotter databases. Abbreviations: OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; DSS, disease-specific survival. DMFS, distant metastasis-free survival. Mb:H, Mutation burden high; Mb:L, Mutation burden low.
(Th1) cells, Th2 cells, regulatory T cells (Tregs), Th17 cells, and exhausted T cells. All results were adjusted based on tumor purity. The results demonstrated a significant positive association between *LPAR2* expression and B cell markers (CD19 and CD79A), M1 macrophage markers (iNOS and IRF5), neutrophil markers (CD11b), Th2 markers (*STAT6* and *STAT5A*), Tfh markers (*BCL6*), and T-cell exhaustion markers (CTLA4) in patients with HNSC (*P* < 0.01, Table 2). In patients with KIRC, a significant positive correlation was found between *LPAR2* expression and CD8+ T cell markers (CD8A and CD8B), total T cell markers (CD3D, CD3E, and CD2), B cell markers (CD19 and CD79A), monocyte markers (CD86 and CD115), TAM markers (CD68 and IL10), M1 macrophage markers (IRF5), M2 macrophage markers (CD163, Vsig4, and M5A4A4A), neutrophil markers (CD11b and CCR7), NK cell markers (KIR2DL4), DC markers (HLA-DPB1, HLA-DRA, HLA-DPA1, and CD11C), Th1 markers (T-bet, STAT4, STAT1, IFN-γ, and TNF-α), Th2 markers (GATA3, STAT6, STAT5A, and IL13), Tfh markers (BCL6 and IL21), Treg markers (FOXP3, CCR8, STAT5B, and TGFβ), and T-cell exhaustion markers (PD-1, CTLA4, and LAG3) (*P* < 0.01, Table 2). However, *LPAR2* expression was negatively correlated with M1 macrophage markers (iNOS), DC markers (BDCA-4), and Treg markers (STAT5B) in KIRC (Table 2).

The results suggested that *LPAR2* expression exhibited a significant correlation with the levels of most markers of B cells, M1 macrophages, Th2 cells, and Tfh cells in patients with HNSC (*P* < 0.0001, Table 2). Strikingly, in patients with HNSC, *LPAR2* expression was closely associated with iNOS of M1 macrophages, *STAT5A* of Th2 cells, and *BCL6* of Tfh cells (*P* < 0.0001, Cor > 0.2, Table 2). In patients with KIRC, the mRNA expression of *LPAR2* was closely correlated with the levels of most markers of total CD8+ T cells (CD8A and CD8B), T cells (CD3D, CD3E, and CD2), B cells (CD19 and CD79A), monocytes (CD86 and CD115), TAMs (CD68), M1 macrophages (IRF5), M2 macrophages (Vsig4), neutrophils (CD11b and CCR7), DCs, Th1 cells (*STAT4*, IFN-γ, TNF-α), Th2 cells (*STAT5A*), Tfh cells (*BCL6*), Tregs (FOXP3, CCR8, and TGF-β), and exhausted T cells (PD-1, CTLA4, and LAG3) (*P* < 0.0001, Cor > 0.2, Table 2). Furthermore, we assessed the relationship between the expression of *LPAR2* and that of the aforementioned markers using GEPIA. The correlation between *LPAR2* expression and these markers was
| Description | Gene markers | HNSC           | KIRC          | Purity | HNSC | KIRC | Purity |
|-------------|--------------|----------------|---------------|--------|------|------|--------|
| CD8+ T cell | CD8A         | 0.035 4.21e-01 | 0.101 2.52e-02 | 0.243  | ***  | 0.201 | ***    |
|             | CD8B         | 0.086 4.88e-02 | 0.144 *       | 0.26   | ***  | 0.226 | ***    |
| T cell (general) | CD3D       | 0.102 2.04e-02 | 0.179 ***     | 0.308  | ***  | 0.271 | ***    |
|             | CD3E         | 0.082 6.03e-02 | 0.159 **      | 0.303  | ***  | 0.263 | ***    |
|             | CD2          | 0.099 2.39e-02 | 0.166 **      | 0.289  | ***  | 0.244 | ***    |
| B cell      | CD19         | 0.185 ***      | 0.256 ***     | 0.354  | ***  | 0.309 | ***    |
|             | CD79A        | 0.157 **       | 0.216 ***     | 0.308  | ***  | 0.265 | ***    |
| Monocyte    | CD86         | 0.009 8.45e-01 | 0.067 1.39e-01 | 0.232  | ***  | 0.215 | ***    |
| TAM         | CCL2         | -0.019 6.65e-01 | 0.036 4.27e-01 | -0.072 | *    | 9.48e-02 | -0.122 | *    |
|             | CD68         | -0.1 2.23e-02  | -0.066 1.43e-01 | 0.227  | ***  | 0.241 | ***    |
| M1 Macrophage | INFN   | -0.063 1.50e-01 | -0.002 9.66e-01 | 0.118  | *    | 0.072 | 1.20e-01 |
|             | IRF5         | 0.173 ***      | 0.18 ***      | 0.301  | ***  | 0.301 | ***    |
| Neutrophils | CD163        | -0.017 6.95e-02 | -0.043 3.38e-01 | 0.057  | 1.92e-01 | 0.057  | 1.92e-01 |
| Natural killer cell | KIR2DL1 | 0.026 5.51e-01 | 0.06 1.83e-01  | -0.06  | 1.67e-01 | -0.061 | 1.94e-01 |
|             | KIR2DL3      | 0.003 8.49e-01 | 0.043 3.46e-01 | 0.005  | 9.00e-01 | 0.024  | 6.13e-01 |
| Dendritic cell | HLA-DPB1 | 0.065 1.40e-01 | 0.135 2.64e-03 | 0.191  | ***  | 0.178 | ***    |
|             | HLA-DOB1     | 0.064 1.44e-01 | 0.108 1.63e-02 | 0.093  | 3.21e-02 | 0.073  | 1.19e-01 |
|             | HLA-DRA      | 0.014 7.52e-01 | 0.078 8.27e-02 | 0.128  | *    | 0.111 | 1.67e-02 |
|             | HLA-DPA1     | 0.023 5.97e-01 | 0.085 5.92e-02 | 0.136  | *    | 0.105 | 2.43e-02 |
| Th1         | T-bet (TBX21) | 0.081 6.35e-02 | 0.142 *       | 0.145  | **   | 0.112 | 1.62e-02 |
|             | STAT4        | 0.088 4.48e-02 | 0.145 *       | 0.297  | ***  | 0.259 | ***    |
|             | STAT1        | -0.052 2.37e-01 | -0.012 7.87e-01 | 0.195  | ***  | 0.154 | **     |
| Th2         | GATA3        | 0.04 3.65e-01  | 0.078 8.50e-02 | 0.174  | ***  | 0.147 | *      |
|             | STAT6        | 0.172 ***      | 0.168 **      | 0.141  | *    | 0.151 | *      |
|             | STAT5A       | 0.23 ***       | 0.255 9.09e-02 | 0.312  | ***  | 0.271 | ***    |
|             | IL13         | 0.075 8.69e-02 | 0.115 1.06e-02 | 0.153  | **   | 0.126 | *      |
| Tfh         | BCL6         | 0.264 ***      | 0.236 ***     | 0.292  | ***  | 0.279 | ***    |
|             | IL21         | 0.001 9.76e-01 | 0.03 5.06e-01 | 0.161  | **   | 0.155 | **     |
similar to that identified using TIMER (Table 3). These findings suggested that LPAR2 was significantly correlated with infiltrating immune cells in HNSC and KIRC and played a significant role in the immune microenvironment of HNSC and KIRC.

Alterations, mutations, methylations, and frequently altered neighbor genes of LPAR2 in patients with HNSC and KIRC

We analyzed genetic alterations of LPAR2 using the cBioPortal for Cancer Genomics in the HNSC and KIRC (TCGA, Firehose Legacy) datasets. LPAR2 mutations and amplifications were found in 3 of 528 patients with HNSC but not in 537 patients with KIRC (Fig. 12A–B). In addition, we calculated the mutations, methylations, mRNA expression Z-scores (RNA Seq V2 RSEM), protein expression Z-scores (RPPA), and putative CNAs of LPAR2 in HNSC using GISTIC (Fig. 12A) and identified the 10 most frequently altered neighbor genes of LPAR2 in HNSC (Fig. 12C). The results revealed that LPAR2 alterations in HNSC were strongly associated with the mutated genes TP53, PVALB, PNKP, LRIT3, ANXA4, EGLN2, SERTAD2, FANCI, UBASH3B, and ZNF253 (Fig. 12C).

Discussion

LPA, a growth factor-like phospholipid, is abundantly found in human tissues and fluids [22]. It participates in various biological functions, such as cell migration, cell proliferation, inflammation, angiogenesis, and survival [27, 28]. LPA acts through G-protein-coupled LPA receptors, which are called LPARs [6, 8]. LPAR2 belongs to the EDG family and contains 351 amino acids [22, 29]. It is unique in the proximal region of the C-terminus and contains several putative palmitoylated cysteine residues and a dileucine motif [30].

A few studies have suggested that LPAR2 is associated with several cancers, such as breast [16, 31, 32], colon [20], ovarian [33], and stomach cancers [17]. These studies have reported that LPAR2 expression is important in cancer biology and may promote gene transcription and cell proliferation in the tumor microenvironment [17, 34, 35]. However, the mechanism of action of LPAR2 in tumors remains unclear.

In addition to traditional cancer treatment, cancer immunotherapy has emerged as an important therapy owing to its adequate efficacy and fewer side effects [36]. Nevertheless, immunotherapy has not been extensively investigated and effectively used to treat patients with HNSC and KIRC [37]. Given that immunotherapy mainly targets the tumor immune microenvironment, we analyzed the effects of LPAR2 on tumor prognosis and immune infiltration of HNSC and KIRC in this study.

We examined the mRNA and protein expression levels of LPAR2 in pan-cancer and the corresponding normal tissues using Oncomine, TIMER, UALCAN, and HPA databases, as well as validated by R software in TCGA and GEO databases. LPAR2 expression was evaluated in tumor and normal tissues in multiple cancer types (Figs. 2 and 4, Table 1). Differences in data collection methods and analytical approaches may

| Table 2 (continued) |
|---------------------|
| Description | Gene markers | HNSC | | | KIRC | | |
| | | Purity | | | Purity | | |
| Cor | P | Cor | P | Cor | P |
| Th17 | STAT3 | 0.056 | 2.01e-01 | 0.054 | 2.32e-01 | 0.029 | 4.99e-01 | -0.003 | 9.41e-01 |
| | IL17A | -0.013 | 7.69e-01 | 0.014 | 7.53e-01 | 0.058 | 1.79e-01 | 0.031 | 5.11e-01 |
| Treg | FOXP3 | 0.08 | 6.9e-02 | 0.133 | * | 0.444 | *** | 0.418 | *** |
| | CCR8 | 0.022 | 6.09e-01 | 0.062 | 1.71e-01 | 0.285 | *** | 0.246 | *** |
| | STAT5B | 0.084 | 5.61e-02 | 0.092 | 4.08e-02 | -0.144 | *** | -0.156 | ** |
| | TGFβ(TGFβ1) | -0.054 | 2.18e-01 | -0.031 | 4.95e-01 | 0.29 | *** | 0.265 | *** |
| T cell exhaustion | PD-1(PDCD1) | 0.11 | 1.19e-02 | 0.174 | ** | 0.378 | *** | 0.357 | *** |
| | CTLA4 | 0.125 | * | 0.199 | *** | 0.364 | *** | 0.333 | ** |
| | LAG3 | 0.099 | 2.40e-02 | 0.152 | * | 0.359 | *** | 0.321 | *** |
| | TIM-3(HAVCR2) | 0.063 | 1.49e-01 | 0.123 | * | -0.009 | 8.44e-01 | -0.022 | 6.34e-01 |
| | GZMB | 0.039 | 3.76e-01 | 0.096 | 3.27e-02 | 0.103 | 1.71e-02 | 0.085 | 6.77e-02 |

Cor, R value of Spearman’s correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. *P<0.01(1e-02); **P<0.001(1e-03); ***P<0.0001(1e-04)

Abbreviations: HNSC Head and Neck squamous cell carcinoma, KIRC kidney renal clear cell carcinoma, TAM tumor-correlated macrophage, Tfh follicular helper T cell, Th T helper cell, Treg regulatory T cell
be attributed to the heterogeneity of \textit{LPAR2} expression among cancer types and databases. However, we consistently observed higher expression of \textit{LPAR2} in HNSC and KIRC across these databases.

We used online tools, such as KM plotter, GEPIA2.0, UACLAN and HPA, and R software to examine the critical role of \textit{LPAR2} in predicting patient outcomes of multiple cancer types in TCGA and GEO databases. Our findings illustrated the expression levels and prognostic value of \textit{LPAR2} in several types of cancers, especially HNSC and KIRC (Figures S1, 2, 3, 4). High \textit{LPAR2} expression was significantly correlated with a worse prognosis in KIRC. However, high \textit{LPAR2} expression was strongly correlated with improved prognosis in HNSC. These contradictory results suggested that \textit{LPAR2} acts as a tumor suppressor gene in HNSC and an oncogene in KIRC.

Given that \textit{LPAR2} expression plays significantly different prognostic roles in HNSC and KIRC, we used UACLAN and KM plotter to examine the relationship between the mRNA expression of \textit{LPAR2} and prognosis in patients with HNSC and KIRC with different clinical characteristics. The findings suggested that high \textit{LPAR2} expression was associated with advanced tumor stages, high tumor grades, and lymph node metastasis in patients with KIRC. Using KM plotter, we found that high \textit{LPAR2} expression was associated with improved prognosis in patients with HNSC with advanced tumor stages and high tumor grades. Meanwhile, high \textit{LPAR2} expression resulted in better prognosis in patients with

\begin{table}[h]
\centering
\caption{Correlation analysis between \textit{LPAR2} and relate genes and immune markers in GEPIA}  
\begin{tabular}{|l|l|l|l|l|l|l|l|l|}
\hline
\textbf{Description} & \textbf{Gene markers} & \textbf{HNSC} & & \textbf{KIRC} & & \\
 & & \textbf{Tumor} & \textbf{Normal} & \textbf{Tumor} & \textbf{Normal} & \\
 & & \textbf{R} & \textbf{P} & \textbf{R} & \textbf{P} & \textbf{R} & \textbf{P} & \textbf{R} & \textbf{P} & \\
\hline
CD8 + T cell & CD8A & -0.012 & 0.79 & 0.059 & 0.7 & 0.26 & *** & 0.027 & 0.82 & \\
 & CD8B & 0.028 & 0.52 & 0.06 & 0.7 & 0.27 & *** & -0.17 & 0.31 & \\
T cell(general) & CD3D & 0.017 & 0.7 & 0.099 & 0.52 & 0.33 & *** & -0.003 & 0.8 & \\
 & CD3E & 0.029 & 0.51 & 0.13 & 0.41 & 0.32 & *** & -0.15 & 0.22 & \\
 & CD2 & 0.038 & 0.13 & 0.4 & 0.33 & 0.3 & *** & -0.12 & 0.33 & \\
B cell & CD19 & 0.13 & 0.2 & 0.19 & 0.75 & 0.35 & *** & -0.15 & 0.2 & \\
 & CD79A & 0.093 & * & 0.34 & * & 0.3 & *** & -0.3 & * & \\
Monocyte & CD86 & -0.017 & 0.7 & 0.23 & 0.14 & 0.24 & *** & -0.06 & 0.62 & \\
 & CD115(CSF1R) & 0.019 & 0.67 & 0.22 & 0.16 & 0.33 & *** & -0.072 & 0.55 & \\
TAM & CD68 & -0.087 & * & 0.3 & 0.051 & 0.16 & *** & -0.06 & 0.62 & \\
M1 Macrophage & INOS(NOS2) & 0.25 & *** & 0.19 & 0.22 & -0.075 & 0.089 & -0.001 & 0.68 & \\
 & IRF5 & 0.16 & *** & 0.57 & *** & 0.35 & *** & -0.049 & 0.68 & \\
M2 Macrophage & Vsig4 & -0.027 & 0.54 & 0.35 & * & 0.27 & *** & 0.0054 & 0.96 & \\
Neutrophils & CD11b(TGAM) & 0.15 & *** & 0.22 & 0.15 & 0.35 & *** & -0.16 & 0.17 & \\
 & CCR7 & 0.054 & 0.22 & 0.14 & 0.36 & 0.28 & *** & -0.034 & 0.77 & \\
Dendritic cell & CD11c(TGAX) & 0.12 & ** & 0.37 & * & 0.43 & *** & -0.12 & 0.33 & \\
Th1 & STAT4 & 0.072 & 0.1 & 0.26 & 0.091 & 0.38 & *** & -0.063 & 0.6 & \\
 & IFN-γ(IFNG) & -0.025 & 0.56 & -0.049 & 0.75 & 0.31 & *** & 0.12 & 0.34 & \\
 & TNF-α(TNF) & 0.084 & 0.055 & 0.068 & 0.66 & 0.28 & *** & 0.27 & * & \\
Th2 & STAT5A & 0.19 & *** & 0.27 & 0.079 & 0.36 & *** & 0.17 & 0.16 & \\
 & BCL6 & 0.26 & *** & 0.4 & ** & 0.33 & *** & 0.78 & *** & \\
 & FOXP3 & 0.087 & * & 0.34 & * & 0.33 & *** & 0.48 & *** & \\
Treg & CCR8 & 0.06 & 0.17 & 0.13 & 0.39 & 0.33 & *** & -0.16 & 0.17 & \\
 & TGFβ(TGFB1) & 0.039 & 0.38 & 0.39 & ** & 0.34 & *** & 0.76 & *** & \\
T cell exhaustion & PD-1(PDCD1) & 0.051 & 0.25 & 0.099 & 0.52 & 0.4 & *** & -0.18 & 0.14 & \\
 & CTLA4 & 0.086 & 0.051 & 0.16 & 0.31 & 0.42 & *** & -0.044 & 0.72 & \\
 & LAG3 & 0.039 & 0.37 & 0.15 & 0.32 & 0.39 & *** & 0.73 & *** & \\
\hline
\end{tabular}
\end{table}

Cor, R value of Spearman’s correlation; * P < 0.05; ** P < 0.01; *** P < 0.001

\textit{Abbreviations:} HNSC Head and Neck squamous cell carcinoma, KIRC kidney renal clear cell carcinoma, TAM Tumor-associated macrophages. Tumor, correlation analysis in tumor tissue of TCGA. Normal, correlation analysis in normal tissue of TCGA.
HNSC, which may be related to their mutational burden status. These results mean that LPAR2 was involved in tumor development and progression of patients with HNSC or KIRC.

Given that high LPAR2 expression affects prognosis related to clinical characteristics in HNSC and KIRC patients, we assessed the relationship between LPAR2 expression and the degree of immune cell infiltration using the TIMER database. Another important finding of this study was that LPAR2 expression was significantly associated with the infiltration of diverse immune cells in HNSC and KIRC. We found that LPAR2 expression had a positive correlation with tumor purity in HNSC and KIRC (Fig. 11A), and the infiltration of B cells, CD4+ T cells, neutrophils, and DCs in KIRC (Fig. 11A). These results suggest that LPAR2 is important for regulating immune cell infiltration in HNSC and KIRC, with particularly strong effects on tumor purity and infiltrating B cells, CD4+ T cells, neutrophils, and DCs.

Furthermore, to investigate the role of LPAR2 in the regulation of tumor immunology in HNSC and KIRC, we analyzed the relationship between LPAR2 expression and marker genes of immune cells. We found a significant positive correlation between LPAR2 expression and the markers of B cells (CD19 and CD79A), M1 macrophages (iNOS and IRF5), neutrophils (CD11b), Th2 cells (STAT6 and STAT5A), Tfh cells (BCL6), and exhausted T cells (CTLA4) in HNSC (P < 0.01, Table 2). In addition, LPAR2 expression was strongly correlated with iNOS of M1 macrophages, STAT5A of Th2 cells, and BCL6 of Tfh cells (P < 0.0001, Cor > 0.2, Table 2). These results indicate that LPAR2 promotes the polarization of macrophages to the M1 phenotype and regulates T cell responses. Furthermore, BCL6 recognizes DNA target sequences similar to those recognized by STAT5 [38]. Some studies have found that STAT5A inhibits cell invasion and metastasis in breast cancer [39]. LPAR2 may play a role in HNSC by interacting with STAT5A and BCL6 via the prolactin–JAK2–STAT5A pathway [38]; but further studies are warranted. In this study, LPAR2 expression was significantly correlated with most immune markers in KIRC, including CD3D and CD3E of total T cells; CD19 and CD79A of B cells; IRF5 of M1 macrophages; STAT5A of Th2 cells; FOXP3 and CCR8 of Treg cells; and PD-1, CTLA4, and LAG3 of exhausted T cells (P < 0.0001, Cor > 0.3, Table 2).
In addition, the results indicate that LPAR2 activates Tregs and B cells, induces T cell exhaustion, and promotes Treg responses to suppress T cell-mediated immunity, thereby regulating T cell responses in KIRC. LPAR2 may promote the polarization of macrophages to the M1 phenotype via IRF5. Therefore, these findings collectively suggest that LPAR2 is a crucial factor for the recruitment and regulation of infiltrating immune cells in HNSC and KIRC.

Conclusion

LPAR2 plays significantly different prognostic roles in HNSC and KIRC, owing to its association with different immune markers. LPAR2 is important for governing immune cell infiltration, and is a valuable prognostic biomarker that may guide treatment in HNSC and KIRC. Nevertheless, further validation experiments are required.

Materials and methods

Data processing and differential expression analysis, survival analysis and correlation analysis

The UCSC Xena dataset was used to acquire TCGA expression and clinical information (https://toil-xena-hub.s3-us-east-1.amazonaws.com/download/TcaTargetGtex_rsem_gene_tpm.gz; Full metadata) [40]. Dataset ID: TcaTargetGtex_rsem_gene_tpm. Raw counts of RNA-sequencing data (level 3) and matching clinical data contains 10,363 tumor tissues and 730 adjacent tissues from 18 types of cancer. Eight independent HNSC and KIRC/RCC gene expression profiles (GSE30784, GSE31056, GSE686, GSE65858, GSE53757, GSE15641, GSE167573 and GSE22541) were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) [41] and processed for analysis. Detailed information of datasets was listed in Table 4. All analytical methods were carried out utilizing the R software version v4.0.3. Expression analysis and Survival curves were drawn using the R packages “ggplot2”, “survival”, and “survminer”[42, 43]. The Log-rank tests as well as the univariate Cox proportional hazards regression generated hazard ratio (HR) and p-values with a confidence interval (CI) of 95% in KM curves.

Oncomine database analysis

The expression data of 715 genes were obtained from 86,733 samples and the mRNA expression levels of LPAR2 in pan-cancer were analyzed using the online cancer microarray database (Oncomine) (www.oncomine.org). The Student’s t-test was used to compare the mRNA expression of LPAR2 between normal and cancer samples. P-value was used to characterize significant differences. The fold change was 1.5, and the cut-off P-value was 0.0001.

TIMER database analysis

The Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) database comprises six tumor-infiltrating immune cell subsets [44], and the expression levels of six subsets are pre-calculated for 10,897 tumors across 32 cancer types from The Cancer Genome Atlas (TCGA). The database allows the analysis of gene expression and tumor immune infiltration (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells [DCs]) in various cancer types. In this study, TIMER was used to analyze the mRNA expression of LPAR2 in various cancer types and investigate the relationship between LPAR2 expression and the degree of infiltration of specific immune cell subsets. Furthermore, differences in the survival of patients with cancer based on gene expression or immune cell infiltration were examined using KM survival analysis. Lastly, the correlation between the expression of LPAR2 and that of specific immune markers was examined.

Table 4 Information of the Selected GEO Datasets

| Datasets   | Contributor         | Disease type | Experimental platform               | Number of cases (cancer/control) |
|------------|---------------------|--------------|-------------------------------------|----------------------------------|
| GSE30784   | Chen C, et al. (2011)| OSCC         | Affymetrix Human Genome U133 Plus 2.0 Array | 167/62                           |
| GSE31056   | Reis PP, et al.(2011)| OSCC         | Affymetrix GeneChip Human Genome HG-U133 Plus 2 Array | 23/24                           |
| GSE686     | Chung CH, et al. (2004) | HNSC        | Agilent Human 1 cDNA Microarray | 78/0                             |
| GSE65858   | Wichmann G, et al(2015) | HNSC        | Illumina HumanHT-12 V4.0 expression beadchip | 270/0                           |
| GSE53757   | von Roemeling CA, et al.(2014) | KIRC      | Affymetrix Human Genome U133 Plus 2.0 Array | 72/72                           |
| GSE15641   | Jones J, et al. (2005) | RCC         | Affymetrix Human Genome U133A Array | 69/23                           |
| GSE167573  | He N, et al.(2021)  | RCC         | HiSeq X Ten (Homo sapiens) | 63/14                           |
| GSE22541   | Wuttig D, et al.(2012) | KIRC      | Affymetrix Human Genome U133 Plus 2.0 Array | 68/0                             |

Abbreviations: OSCC oral squamous cell carcinoma, HNSC Head and Neck squamous cell carcinoma, KIRC kidney renal clear cell carcinoma, RCC renal cell cancer
UALCAN
UALCAN (http://ualcan.path.uab.edu/index.html) is an interactive web resource used for analyzing publicly available cancer omics data (TCGA, MET500, and Clinical Proteomic Tumor Analysis Consortium) [45]. In this study, UALCAN was used to examine the mRNA expression level of LPAR2 in different cancer and normal samples using the TCGA data and investigate the relationship between LPAR2 expression and different clinical characteristics. In addition, the prognostic value of LPAR2 in pan-cancer and the relationship between LPAR2 expression and the prognosis of patients with different clinical characteristics were analysed.

KM plotter analysis
The KM plotter (http://kmplot.com/analysis/) is an online database, which contains microarray gene expression data and survival information derived from the European Genome-Phenome Archive, Gene Expression Omnibus (GEO), and TCGA. It is used to assess the influence of multiple genes on the survival rate in 21 cancer types in a large number of samples [46]. In this study, the KM plotter was used to analyze the prognostic value of LPAR2 in pan-cancer and investigate the relationship between LPAR2 expression and the prognosis of patients with different clinical characteristics.

GEPIA2 database analysis
GEPIA (http://geopia.cancer-pku.cn/index.html) uses standard processing pipelines to analyze the RNA-sequencing expression data of 8,587 normal samples and 9,736 tumors from the GTEx and TCGA projects [47]. GEPIA2 (http://gepia2.cancer-pku.cn/#index) is an updated version of GEPIA [48]. In this study, GEPIA2 was used to examine the relationship between the mRNA expression of LPAR2 and pan-cancer prognosis as well as the relationship between the expression of LPAR2 and that of the markers of immune cell infiltration.

HPA database
The Human Protein Atlas (HPA) database (www.proteinatlas.org) was used to analyze the protein expression of LPAR2 in HNSC, KIRC, and normal tissues [49, 50]. HPA provides access to the protein expression profiles of 32 human tissues and uses antibody profiling to accurately assess protein localization. In addition, it provides the measurements of RNA levels. In this study, HPA was used to visualize the representative immunohistochemical images of LPAR2 in HNSC, KIRC, and their corresponding normal tissues. In addition, the relationship between the protein expression level of LPAR2 and the prognosis of patients with HNSC and KIRC was examined.

TCGA and cbioPortal for cancer genomics
The cbioPortal for Cancer Genomics tool (http://www.cbioportal.org) is used to analyze, visualize, and download cancer genomics datasets [51]. In this study, the cbioPortal for Cancer Genomics was used to download the HNSC and KIRC (TCGA, Firehose Legacy) datasets for LPAR2 analysis, which contained histopathological data of 528 patients with HNSC and 537 patients with KIRC. The genomic profiles were evaluated via the Genomic Identification of Significant Targets in Cancer (GISTIC) analysis and included the assessment of mutations, methylations, mRNA expression z-scores (RNA Seq V2 RSEM), protein expression z-scores (RPPA), and putative copy number alterations (CNAs). Co-expression was evaluated according to the instructions provided on cbioPortal.

Statistical analysis
Data were analyzed using the log-rank test, which included fold change, hazard ratio (HR), and P-values. Furthermore, the degree of relationship between specific variables was measured via Spearman’s correlation analysis, with R values, to measure the relationship strength as follows: “very weak”, 0.00–0.19; “weak”, 0.20–0.39; “moderate”, 0.40–0.59; “strong”, 0.60–0.79; and “very strong”, 0.80–1.0. A P-value < 0.05 indicated statistical significance.

Abbreviations
LPAR2: Lysophosphatidic acid receptors 2; TIMER: Tumor Immune Estimation Resource; HPA: Human Protein Atlas; GEPIA: Gene Expression Profiling Interactive Analysis; TCGA: The Cancer Genome Atlas; GTEx: The Genotype-Tissue Expression; ONCOMINE: Online cancer microarray database; K-M plotter: Kaplan-Meier plotter; HNSC: Head and neck squamous cell carcinoma; KIRC: Kidney renal clear cell carcinoma; ATX: Autotaxin; BC: Breast cancer; RNA Seq V2 RSEM: MRNA expression z-scores; RPPA: Protein expression z-scores; CNA: Copy-number alterations; ECL: Extracellular loop; EDG: Endothelial differentiation gene; GEO: Gene Expression Omnibus; GEPIA: Gene Expression Profiling Interactive Analysis; GPCRs: G-protein coupled receptors; HR: Hazard ratio; LP: Lysophospholipid; LPA: Lysophosphatidic acid; LPARs: Lysophosphatidic acid receptors; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; KICH: Kidney chromophobe tumour; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; ESCA: Esophageal carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; COAD: Colon adenocarcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; STAD: Stomach adenocarcinoma; DLD1: DLD1 colon adenocarcinoma; HCC1954: Hepatocellular carcinoma; UCEC: Uterine corpus endometrial carcinoma; CHOL: Cholangio carcinoma; GBM: Glioblastoma multiforme; PAAD: Pancreatic adenocarcinoma; THYM: Thymoma; THCA: Thyroid carcinoma; ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; LPCA: Prostate adenocarcinoma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; UCSC: Uterine corpus endometrial carcinoma; CHOL: Cholangiocarcinoma; THY: Thyroid carcinoma; CES: Cervical squamous cell carcinoma; THYM: Thymoma; ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; PCPG: Pheochromocytoma and Paraganglioma; UCEC: Uterine corpus endometrial carcinoma; UCSC: Uterine Carcinoma sarcoma; OES: Overall survival; DFS: Disease-free survival; DFS: Disease-free survival; RFS: Relapse-free survival; DSS: Post-progression survival; DSS: Disease-specific survival; DMFS: Distant metastasis-free survival; F_P: First progression; TAM: Tumor-associated macrophages; NK cells: Natural killer cells; DCs: Dendritic cells; Th: Follicular helper T cell; T: T helper cell; HR: Hazard ratio; OSCC: Oral squamous cell carcinoma; RCC: Renal cell cancer.
Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41065-022-00229-w.

Additional file 1: Table S1. Clinical characteristics of patients in HPA.
Additional file 2: Table S2. Clinical characteristics of patients with HNSC in HPA.
Additional file 3: Table S3. Clinical characteristics of patients with KIRC in HPA.
Additional file 4: Figure S1. Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancers in the Kaplan-Meier plotter databases (A-H).
Additional file 5: Figure S2. Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancer in UACLAIN databases (A-S).
Additional file 6: Figure S3. Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancer in TCGA databases (A-Q).
Additional file 7: Figure S4. Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancer in GEPIA databases (A-Q).
Additional file 8: Figure S5. Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in HNSC and KIRC from GEO databases and the paired ROC curves of measuring the predictive value (A-D).

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Authors’ contributions

KS and ZL performed the analysis of the data. KS and RC wrote the manuscript. KS and JL designed the study. All authors read and approved the manuscript.

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Availability of data and materials

All the datasets were retrieved from the publishing literature, so it was confirmed that all written informed consent was obtained.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent for the publication of this work.

Competing interests

The authors declare that they have no competing interests.

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