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Kai Sun

People's Hospital of Liuzhou

Rixin Chen

People's hospital of Liuzhou

Jingzhang Li

People's hospital of Liuzhou

Zhanxiong Luo (✉ luozhaoxianglz@163.com)

People's Hospital of Liuzhou <https://orcid.org/0000-0002-3741-4691>

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

Kai Sun, MD^{1*}, Rixin Chen, MD¹, Jingzhang Li, MD¹, Zhanxiong Luo, MD^{1}**

¹Department of oncology, People's Hospital of Liuzhou, Liuzhou 545001, Guangxi Zhuang Autonomous Region, China.

**Corresponding author: Dr. Zhanxiong Luo, Department of oncology, People's Hospital of Liuzhou, Liuzhou 545001, Guangxi Zhuang Autonomous Region, China.

Tel./fax: +86 07722662134. E-mail address: luozhaoxianglz@163.com

Keywords: head and neck squamous cell carcinoma, kidney renal clear cell carcinoma., prognosis, LPAR2, tumor infiltration

Abstract

Background: LPA and its receptors represent two key players in regulating cancer progression. Recent findings suggest that upregulation of lysophosphatidic acid receptor 2(LPAR2) may play a role in carcinogenesis. But there are few studies on the relationship between LPAR2 and tumor immune microenvironment.

Methods: In this study, we analyzed LPAR2 expression in pan tumors via the Oncomine, Tumor Immune Estimation Resource (TIMER), and UALCAN. We investigated the influence of LPAR2 on clinical prognosis from Kaplan-Meier plotter (K-M plotter), Gene Expression Profiling Interactive Analysis (GEPIA), UALCAN and Human Protein Atlas (HPA). We also examined the relationship of

expression levels of LPAR2 and clinical and molecular criteria in HNSC and KIRC by UALCAN. Then we explored the relationship between LPAR2 expression and prognosis in HNSC and KIRC patients with different clinical characteristics via K-M plotter. The correlations between LPAR2 and cancer immune infiltrates was examined via TIMER. In addition, correlations between the expression of LPAR2 and gene markers of immune infiltrates were analyzed by TIMER and GEPIA. We also used the cBioPortal to calculate mutations, methylations and altered neighbor genes of LPAR2.

Results: We found that LPAR2 different expression was significantly related with the outcome of multiple types of cancer from The Cancer Genome Atlas (TCGA), particularly in head and neck squamous cell carcinoma (HNSC) and kidney renal clear cell carcinoma (KIRC). Furthermore, high expression levels of LPAR2 were found to be significantly associated with a variety of immune markers in particular immune cell subsets in HNSC and KIRC.

Conclusions: Our finding indicates that high LPAR2 expression playing significantly different prognostic roles in HNSC and KIRC, might be due to associate with different immune markers. And LPAR2 is correlated with tumor immune cell infiltration and is a valuable prognostic biomarker in HNSC and KIRC patients.

1 | INTRODUCTION

Lysophosphatidic acid (LPA, 1-acyl-2-hemolyticsn-glycerin-3-phosphate) is a bioactive glycerophosphatidic acid, naturally occurring lysophospholipid (LP), widely exists in human body^[1]. lysophospholipids (LPS, LPE, and LPC) are hydrolyzed by autotaxin (ATX) to produce LPA in plasma, serum and adipocytes^[2]. LPA acts as a growth factor by activating distinct high affinity G protein-coupled receptors (GPCR), such as promoting cell growth, differentiation, migration, division and survival on many cell types^[3,4]. LPA has several G-protein coupled receptors, named as lysophosphatidic acid receptors (LPARs)^[5]. According to their homology, LPARs can be subdivided into six types: LPAR1-6. LPAR1-6 can be divided into two main subfamilies with obvious difference: endothelial differentiation gene family (LPAR1–3) and purinergic receptor family (LPAR4–6)^[6]. LPAR1-3 belongs to the endothelium differentiation gene (EDG) receptor, meanwhile LPAR4-6 is a non EDG receptor^[7]. LPA receptors have seven transmembrane domains, three intracellular loops and three extracellular loops^[8]. Now it's clear that the LPA receptors signal pathway produce different results under different environments and cell types involved at least two G α subunits (G α q/11, G α 12/13, G α i/o and G α S) activating different downstream pathways^[9, 10]. Several signaling pathways, such as RhoA pathway, phospholipase C pathway, PI3K/PAK1/ERK pathway, Ras-Raf-MEK-ERK pathway, and Rac pathway are activated by G α q/11, G α 12/13, G α i/o and G α S^[9, 11]. Due to the similar G protein types, six LPA receptors have similar biological functions^[12]. Multiple studies have revealed the key role of LPA and its receptors in various cancer tissues, such as breast cancer, lung cancer, liver cancer, pancreatic cancer, ovarian cancer, neuroblastoma, and thyroid cancer^[13, 14].

Although there are many studies on the expression and function of LPAR1 and LPAR3 in several tumors, the research of LPAR2 is fewer. Several studies find that LPAR2 is aberrantly expressed in

several tumors, such as breast cancer, colorectal cancer, kidney cancer and pancreatic cancer^[15-18]. Some research reveal LPAR2 can promote a robust activation of RhoA to mediate cell migration^[19]. Recent report indicates LPAR2 can regulate the cell–cell adhesion level of neural crest cells by internalization of N-cadherin downstream^[20]. A literature also reports LPAR2 is significantly associated with LPA-induced IL-6 and IL-8 expression, which promoted BC progression^[21]. However, the mechanisms of action of the LPAR2 in tumors appear diverse and are not well understood.

In this study, we systematic investigated the expression of LPAR2 and its relationship with prognosis of pan tumors via the Oncomine, Tumor Immune Estimation Resource (TIMER), UALCAN, GEPIA, Human Protein Atlas (HPA), and K-M plotter. We examined the relationship of expression of LPAR2 and clinical and molecular criteria in by UALCAN. Then we explored the relationship between LPAR2 expression and HNSC and KIRC patient prognosis with different clinical characteristics via K-M plotter. Next, we analyzed the correlation of LPAR2 and tumor-infiltrating immune cells in microenvironments of pan tumors via TIMER and GEPIA. Finally, we used the cBioPortal online tool to analyze alterations, mutations, methylations and pathways of LPAR2. This study showed a potential LPAR2 expression mechanism and different prognostic roles in HNSC and KIRC, LPAR2 is a key factor in HNSC and KIRC immune microenvironment.

2 MATERIAL S AND METHODS

Oncomine database analysis

The online cancer microarray database (ONCOMINE) gene expression array dataset (www.oncomine.org) compiled 715 gene expression data in 86,733 samples. We analyzed the mRNA expression levels of LPAR2 in pan cancers by ONCIMINE. The Student's t test was used to compare the mRNA expression of LPAR2 in different normal specimens and that in cancers

specimens, P value for difference. The fold change was 1.5. The cut-off of P value was defined as 0.0001.

TIMER database analysis

TIMER (<https://cistrome.shinyapps.io/timer/>) database comprise six tumor-infiltrating immune subsets^[22]. The levels of six subsets are precalculated for 10,897 tumors across 32 cancer types from The Cancer Genome Atlas (TCGA). The database analyzed gene expression and tumor immune infiltration (B cells, CD4+ T cells, CD8+ T cells, Neutrophils, Macrophages and Dendritic cells) in various cancers. We used TIMER to analyze the mRNA expression of LPAR2 in various cancers, and we explored the relationship between this LPAR2 expression and the degree of infiltration by the specific immune cell subsets. We next explored the difference in survival of cancer patients with gene expression or immune cell infiltration by Kaplan-Meier survival analysis. Finally, we evaluated how LPAR2 expression associated with the expression of specific immune infiltrating cell subsets markers.

UALCAN

UALCAN (<http://ualcan.path.uab.edu/index.html>) is an interactive web resource for analyzing cancer OMICS data^[23]. It comprises publicly available cancer OMICS data (TCGA, MET500 and CPTAC). We used UALCAN to study the mRNA expression levels of LPAR2 in different cancers specimens and that in normal specimens in TCGA database and the relationship between the expression and different clinical characteristic. Then we analyzed the prognostic values of LPAR2 in pan cancers by UALCAN, and the relationship between the expression of LPAR2 and prognosis in patients with different clinical characteristic.

Kaplan-Meier plotter analysis

Kaplan-Meier plotter(KM plotter; <http://kmplot.com/analysis/>) is an online database, which containing microarray gene expression data and survival information derived from European

Genome-Phenome Archive, Gene Expression Omnibus and TCGA, offering a way to explore the influence of multiple genes on the survival rate of 21 different types of cancers in a large number of samples for different cancers cohorts^[24]. We used Kaplan-Meier plotter to analyze the prognostic values of LPAR2 in pan cancers. We also explored the relationship between the expression of LPAR2 and prognosis in patients with different clinical characteristic.

GEPIA2 database analysis

Gene Expression Profiling Interactive Analysis (GEPIA) is using standard processing pipelines to analyze the RNA sequencing expression data of 8,587 normal samples and 9,736 tumors from GTEx and TCGA projects. GEPIA2 is a updated version of GEPIA , Via GEPIA2, we assessed the relationship between the mRNA expression levels of LPAR2 and patient prognosis in pan cancers, and the link between expression of LPAR2 with the expression of immune cell infiltration particular markers of tumors.

HPA database

We used the human protein atlas database (HPA) (www.proteinatlas.org) to analyze protein expression of LPAR2 between HNSC tissues, KIRC tissues with their corresponding normal tissues^[25]. The HPA provides access to 32 human tissues and their protein expression profiles and uses antibody profiling to accurately assess protein localization. Additionally, the HPA provides measurements of RNA levels. In this study, representative immunohistochemistry images of different LPAR2 in HNSC and KIRC tissues and corresponding normal tissues were directly visualized by HPA, and we assessed the relationship between the protien expression levels of LPAR2 and patient prognosis in HNSC and KIRC cancers.

TCGA data and cBioPortal

The cBioPortal for Cancer Genomics provides analysis, visualization, and downloading of cancer genomics datasets^[26]. By using the cBioPortal for Cancer Genomics (www.cbioportal.org), the HNBC dataset (TCGA, Firehose Legacy) and the KIRC dataset (TCGA, Firehose Legacy), which contains including histopathological data of 528 HNBC patients and 537 KIRC patients, was selected for LPAR2 analysis. The genomic profiles included mutations, methylations, mRNA expression z-scores (RNA Seq V2 RSEM), protein expression Z-scores (RPPA) and putative copy-number alterations (CNA) from GISTIC. Co-expression were calculated according to the online instructions of cBioPortal.

Statistical analysis

We analyzed data by a log-rank test, such as fold-change, Hazard ratio(HR), and P-values. We measured the degree of relationship between specific variables via Spearman's correlation analysis, with the r values to measures the relationship strength: 'very weak': 0.00–0.19, 'weak': 0.20–0.39, 'moderate':0.40–0.59, 'strong': 0.60–0.79, 'very strong': 0.80–1.0. $P < 0.05$ was the threshold of significance.

3 RESULTS

3.1 Assessment of LPAR2 expression in different cancers and normal tissues

First, via the Oncomine database, we explored the mRNA expression levels of LPAR2 in pan tumors and normal tissue types. The results showed that in some tumors, such as bladder cancer, brain and CNS cancer, breast cancer, colorectal cancer, kidney cancer, lung cancer and lymphoma, the expression levels of LPAR2 were higher than normal tissue control (Figure 1). In kidney cancer, leukemia, lung cancer, lymphoma and sarcoma tissues, LPAR2 expression was lower than normal

tissue controls (Figure 1). Table 1 summarizes the detailed findings of specific tumor types. We further assessed how LPAR2 expression differs in pan tumor types in TCGA databases via TIMER. LPAR2 expression was significantly higher in Bladder Urothelial Carcinoma(BLCA), Breast invasive carcinoma(BRCA), Cholangiocarcinoma(CHOL), Colon adenocarcinoma(COAD), Esophageal carcinoma(ESCA), Head and Neck squamous cell carcinoma(HNSC), Kidney renal clear cell carcinoma(KIRC), Liver hepatocellular carcinoma(LIHC), Lung adenocarcinoma(LUAD), Lung squamous cell carcinoma(LUSC), Prostate adenocarcinoma(PRAD), Rectum adenocarcinoma(READ), Stomach adenocarcinoma(STAD) and Uterine Corpus Endometrial Carcinoma(UCEC) compared with adjacent normal tissues(Figure 2). However, LPAR2 expression were significantly lower in Kidney Chromophobe (KICH) and Thyroid carcinoma (THCA) compared with adjacent normal tissues (Figure 2). Then we examined LPAR2 expression using TCGA databases by UALCAN, the mRNA expression levels of LPAR2 were significantly higher than normal controls in BLCA, BRCA, Cervical squamous cell carcinoma and endocervical adenocarcinoma (CECS), Glioblastoma multiforme (GBM), HNSC, KIRC, Kidney renal papillary cell carcinoma (KIRP), LIHC, LUAD, LUSC, PRAD, READ, STAD and UCEC (Figure 3). In contrast, the expression of LPAR2 was significantly lower than normal control tissues in KICH, THCA (Figure 3). Differences between the expression of LPAR2 in tumors and normal adjacent tissue samples are shown in Figure 3.

3.2 The relationship between the expression of LPAR2 and cancer patient prognosis

We employed the Kaplan-Meier plotter database to explore correlation between the expression levels of LPAR2 and the survival of patients in pan tumors and normal tissue types (Figure S1). A few cancer types, such as BLCA, BRCA, CESC, HNSC, KIRC, STAD, THYM and UCEC, exhibited a significant correlation between LPAR2 expression levels and patient prognosis, (Figure 4) .We

revealed that higher LPAR2 expression levels have significantly relationship with poorer prognosis in BRCA (overall survival (OS), HR = 1.42 (1.16 – 1.74), P = 0.00069; relapse free survival (RFS), HR = 0.8 (0.71 – 0.89), P = 7.3e–05; 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; post-progression survival (PPS), HR = 1.33 (1.06 – 1.67), P = 0.014) and with poorer OS in KIRC (OS, HR = 2.44 (1.8 – 3.31), P = 3.5e–09) (Figure 4 B, D, H, I, J, G). On the contrary, we found that high expression levels of LPAR2 were associated with improved OS in 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), TYHM (OS HR = 0.17 (0.04 – 0.68), P = 0.0046), UCEC (OS HR = 0.59 (0.38 – 0.9), P = 0.014) and with improved RFS in BRCA (RFS, HR = 0.8 (0.71 – 0.89), P = 7.3e–05), UCEC (RFS, HR = 0.54 (0.32 – 0.91), P = 0.018) (Figure 4A, E, F, K, L, C, M). But there were no significant correlations between the mRNA expression levels of LPAR2 and patient prognosis in other cancers (Figure S1). Then we assessed the relationship between LPAR2 expression levels and patient prognosis in multiple cancer types via the GEPIA database (Figure S2). We found that high mRNA expression levels of LPAR2 were associated with poorer prognosis in KIRC (OS HR = 2.1, P = 3.6e–06; disease free survival (DFS), HR = 1.9, P = 9e–04), and with poorer OS in PRAD (OS, HR = 7.7, P = 0.024), poorer DFS in CHOL (DFS, HR = 2.6, P = 0.048) (Figure 5 C, D, E, A). Meanwhile, high mRNA expression levels of LPAR2 were correlated with better OS in HNSC (OS HR = 0.71, P = 0.012) and THYM (OS HR = 0.11, P = 0.013) (Figure 5 B, F), but show no significant correlation in BRCA (OS HR = 0.85, P = 0.49; DFS HR = 0.74, P = 0.29) and other tumors (Figure S2). Our findings revealed the expression and prognostic value of LPAR2 in several types of cancers, especially in HNSC and KIRC. It was noteworthy that high LPAR2 expression playing significantly different prognostic roles in HNSC and KIRC.

3.3 The relationship between the protein expression of LPAR2 and cancer patient prognosis in HNSC and KIRC patients

After analyzing the LPAR2 mRNA expression and the relationship between its expression and cancer patient prognosis in HNSC and KIRC, we explored the protein expression of LPAR2 and correlations between LPAR2 protein expression levels and prognosis in HNSC and KIRC by the Human Protein Atlas (HPA). As shown in Figure 6, we indicated medium protein expression of LPAR2 in HNSC and KIRC tissues, and low protein expression in corresponding normal tissues by HPA. Then we assessed the relationship between LPAR2 protein expression levels and patient prognosis in HNSC and KIRC tissues via the HPA. As same as the relationship of LPAR2 mRNA expression and prognosis, we found that high protein expression levels of LPAR2 were associated with poorer OS in KIRC ($P = 3.5e-9$), but with improved OS in HNSC ($P = 0.0023$) (Figure 7 B, A).

3.4 The relationship between the LPAR2 mRNA expression and clinical characteristics of HNSC and KIRC patients

Since LPAR2 expression playing significantly different prognostic roles in HNSC and KIRC, we employed TCGA databases in order to examine the varied expression levels of LPARs with different clinical and molecular criteria in HNSC and KIRC patients by UALCAN. For the criterion of tumor stage, we found that significant upregulated expression of LPAR2 in stage 1-4 compared normal group in HNSC patients ($P < 0.001$) (Figure 8A). For the criterion of race, there was higher mRNA expression level of LPAR2 in caucasian and african-american HNSC patients than control group ($P < 0.001$), but there was no significant difference existed between asian group and control group of LPAR2 expression ($P > 0.05$) (Figure 8B). Upregulated expression levels of LPAR2 were found in both of female and male HNSC patients compared normal group ($P < 0.001$) (Figure 8C). Then, we found upregulated expression of LPAR2 in 21-40 years old ($P < 0.001$), 41-60 years old ($P < 0.001$), 61-80 years old groups and 81-100 years old groups ($P < 0.001$) compared control group in HNSC patients (Figure 8D). The

findings suggested that the mRNA levels of RNA were significant upregulated in HNSC patients than normal group in spite of tumor grade, HPV expression status, nodal metastasis status and mutation status ($P < 0.01$, $P < 0.001$) (Figure 8E, F, G, H.).

In KIRC patients' group, upregulated expression of LPAR2 was found in tumor stage 3 and 4 group ($P < 0.001$) (Figure 9A). But there was no significant difference existed in tumor stage 1 and 2 group compared normal group ($P > 0.05$) (Figure 9A). Same as in HNSC, we found that significant upregulated expression of LPAR2 caucasian and african-american KIRC patients than control group ($P < 0.001$), but there was no significant difference existed between asian group and control group of LPAR2 expression ($P > 0.05$) (Figure 9B). Upregulated expression of LPAR2 were found in both of female and male KIRC patients compared normal group ($P < 0.001$) (Figure 9C). Next, we found upregulated expression of LPAR2 in 21-40 years old ($P < 0.05$), 41-60 years old ($P < 0.01$) and 61-80 years old group ($P < 0.001$) compared control group, but not in 81-100 years old groups ($P > 0.05$) in KIRC patients (Figure 9D). Then the findings suggested the mRNA expression of LPAR2 levels were higher in KIRC patients of tumor grade 3,4 compared to normal group ($P < 0.001$), but there was no significant difference existed between tumor grade 1,2 group and control group of LPAR2 expression ($P > 0.05$) (Figure 9E). Positive nodal patients with KIRC showed higher mRNA expression level of LPAR2 than negative nodal patients, whereas the expression of LPAR2 was higher in both negative nodal patients and positive nodal patients than normal group ($P < 0.01$, $P < 0.001$) (Figure 9F). These findings suggested the expression level of LPAR2 was associated with tumor stage, tumor grade and lymph node metastasis in KIRC patients, and LPAR2 expression levels might have relationship with race in HNSC and KIRC patients.

3.5 the relationship between the LPAR2 mRNA expression and prognosis in HNSC and KIRC patients with different clinical characteristics

For better understand the mechanisms of LPAR2 expression in HNSC and KIRC, we investigated the relationship between the LPAR2 mRNA expression and prognosis in HNSC and KIRC patients

with different clinical characteristics in TCGA databases via Kaplan-Meier plotter. Higher mRNA expression levels of LPAR2 were associated with better OS in tumor stage 2 to 4 of HNSC patients (stage2 HR = 0.45(0.2-0.99), P = 0.042; stage 3 HR = 0.35(0.14-0.88), P = 0.019; stage4, HR= 0.55(0.38-0.79), P = 0.00094), but no significant difference existed in tumor stage 1 group (P > 0.05) (Figure 10 A, B, C, D). Overexpression of LPAR2 was correlated with better OS in HNSC male patients (HR=0.58(0.42-0.81), P=0.0012), but no significant difference in female group (P=0.5) (Figure 10 E, F). For the criterion of race, higher mRNA expression level of LPAR2 showed better OS in white patients (HR=0.64(0.47-0.86), P=0.0032), not in black/asia patients (P>0.05) (Figure 10 G, H). Then we found that upregulated mRNA expression levels of LPAR2 were associated with improved OS in tumor 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) of HNSC patients (Figure 10 J, K). But this relationship did not exist in tumor grade 1 of HNSC patients (P>0.05) (Figure 10 I). For the criterion of mutation status, the results indicated that high mRNA expression level of LPAR2 was correlated with improved OS in LPAR2 mutation burden low group of HNSC patients (HR=0.46(0.29-0.74), P=0.00095) (Figure 10 M). But in LPAR2 mutation burden high group, there was no significant relationship between the mRNA expression level of LPAR2 and prognostic status (P>0.05) (Figure 10 L).

In KIRC patients' group, upregulated expression of LPAR2 was associated with worse OS in tumor stage 1 (HR=2.07(1.08-3.97), P=0.024), tumor stage 3 (HR=2.42(1.08-5.41), P=0.026) and tumor stage 4 group (HR=1.85(1.04-3.31), P=0.034) (Figure 11 A, C, D), meanwhile there was no significant relationship existed in tumor stage 2 group compared normal group (P>0.05) (Figure 10 B). Specifically, high LPAR2 mRNA expression was associated with shorter OS in white patients (HR= 2.55(1.86-3.51), P=2.1e-09), but was not associated with OS in black/asia patients (P>0.05) (Figure 11 J, I). Overexpression of LPAR2 was associated with worse OS in male (HR=2.76(1.88-4.04), P=5.7e-08) and female (HR=3.83(2-7.36), P=1.4e-05) KIRC patients (Figure 11 G, H). In addition, high LPAR2 expression

was associated with worse OS in tumor grade 2 to 4 of KIRC patients (grade 2, HR=2.94(1.31-6.6), P=0.0062; grade 3, HR=2.72(1.46-5.05), P=0.001; grade 4, HR=1.75(1.02-3.03), P=0.041) (Figure 11 K, L, M). Higher mRNA expression levels of LPAR2 were associated with worse OS in LPAR2 mutation burden high group and low group of KIRC patients (high, HR = 2.15(1.23-3.74), P = 0.0058; low, HR = 3.01(1.33-6.83), P = 0.056) (Figure 11 N, O).

These results suggested that LPAR2 expression levels could impact the prognosis in HNSC patients with tumor stage and grade status, but had no significant difference in KIRC patients. Upregulated expression levels of LPAR2 brought good outcome in male HNSC patients or LPAR2 mutation burden low HNSC patients. In addition, LPAR2 expression levels were significant associated with prognosis in white HNSC and KIRC patients.

3.6 The expression of LPAR2 associated with immune cell infiltration in HNSC and KIRC.

Tumor-infiltrating lymphocytes are independent predictors of tumor stage, grade and lymph node status in cancers^[27, 28]. Therefore, we used the TIMER database to explore the relationship between LPAR2 expression and the degree of immune cell infiltration in HNSC and KIRC (Figure 12). Our finding suggested that LPAR2 expression was significantly correlated with tumor purity levels (R = 0.2, P = 7.74e-06), B cells level (R = 0.217, P = 1.70e-05), CD4+T cells level (R = 0.149, P = 1.07e-03), , whereas there was no relationship with CD8+T cells, macrophages, neutrophils and dendritic cells (DCs) levels in HNSC (Figure 12A). In KIRC, the expression levels of LPAR2 had significantly relationship with tumor purity levels (R = -0.155, P = 8.49e-04), B cells level (R = 0.168, P = 2.94e-04), CD4+ T cells level (R = 0.242 P = 1.46e-07) , neutrophils level (R = 0.197 P = 2.09e-05) and dendritic cells level (R = 0.141, P = 2.66e-03) (Figure 12 A). Meanwhile, there were no significant associations between LPAR2 levels and CD8+T cell and macrophages in KIRC (Figure 12 A). We further

investigated the correlation between LPAR2 expression levels and immune cell infiltration in HNSC and KIRC by generating KM plots via the TIMER database. The results showed that B cells level infiltration significantly correlated with HNSC prognosis ($P=0.045$) (Figure 12B), meanwhile significant association between mRNA expression of LPAR2 and prognosis was observed in KIRC ($P<0.001$) (Figure 12B). These results indicate that LPAR2 plays an important role in the regulation of immune cell infiltration in HNSC and KIRC. In HNSC, LPAR2 has a particularly strong role in tumor purity and the infiltration of B cells, CD4+ T cells. Meanwhile LPAR2 is a pivotal player in tumor purity and the infiltration of B cells, CD4+ T cells, neutrophils and dendritic cells.

3.7 The relationship between LPAR2 and immune marker expression

Since LPAR2 plays an important role in the regulation of immune cell infiltration in HNSC and KIRC, we explored the relationship between the expression of LPAR2 and immune cell infiltration by the TIMER and GEPIA databases, basing on immunological markers sets in HNSC and KIRC. Meanwhile, we evaluated the relationship between LPAR2 expression and several immunological markers subsets including total T cells, B cells, CD8+ T cells, TAMs, monocytes, M1 and M2 macrophages, natural killer cells (NK cells), neutrophils, dendritic cells (DCs), Tfh cells, Th1 cells, Th2 cells, Tregs, Th17 cells and exhausted T cells. All results were adjusted with tumor purity. The finding showed significant positive associations between LPAR2 expression and B cells (CD19, CD79A), M1 macrophage markers (INOS, IRF5), neutrophils markers (CD11b), Th2 markers (STAT6, STAT5A), Tfh markers (BCL6), T cell exhaustion markers (CTLA4) in HNSC ($P<0.01$, Table 2). In KIRC, the finding showed significant positive correlations between LPAR2 expression and CD8+T cell (CD8A, CD8B), total T cells (CD3D, CD3E, CD2), B cells (CD19, CD79A), monocyte markers (CD86, CD115), TAM markers (CD68, IL10), M1 macrophage markers (IRF5), M2 macrophage markers (CD163, VSIG4, MS4A4A), neutrophils markers (CD11b, CCR7), NK cell markers (KIR2DL4), DC markers (HLA-DPB1, HLA-DRA, HLA-DPA1, CD11C), Th1 markers (T-

bet, STAT4, STAT1, IFN- γ , TNF- α), Th2 markers (GATA3, STAT6, STAT5A, IL13), Tfh markers (BCL6, IL21), Treg markers (FOXP3, CCR8, STAT5B, TGF β), T cell exhaustion markers (PD-1, CTLA4, LAG3) in KIRC (P<0.01, Table 2). Conversely, the expression level of LPAR2 was negative associated with M1 macrophage markers (INOS), DC markers (BDCA-4), Treg markers (STAT5B) in KIRC (Table 2).

The results suggested that LPAR2 expression had significant relationships with the levels of most markers of B cells, M1 macrophages, Th2, Tfh in HNSC (P<0.0001, Table 2). Remarkably, LPAR2 expression had closely relationship with INOS of M1 macrophage, STAT5A of Th2 and BCL6 of Tfh (P < 0.0001, Cor > 0.2, Figure 14A). In KIRC, the mRNA expression levels of LPAR2 had significantly associations with the levels of most markers of total CD8+ T cells, T cells, B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, DCs, Th1 cells, Th2 cells, Tfh cells, Tregs and exhausted T cells (P<0.0001, Table 2). Intriguingly, LPAR2 expression had closely relationship with CD8A, CD8B of CD8+T cell, CD3D, CD3E, CD2 of total T cells, CD19, CD79A of B cells, CD86, CD115 of monocytes, CD68 of TAM, IRF5 of M1 macrophages, VSIG4 of M2 macrophage markers, CD11b, CCR7 of neutrophils, STAT4, IFN- γ , TNF- α of Th1, STAT5A of Th2 markers, BCL6 of Tfh, FOXP3, CCR8, TGF β of Treg markers, PD-1, CTLA4, LAG3 of T cell exhaustion markers in KIRC (P<0.0001, Cor>0.2, Table 2). Next, we explored the relationship between expression of LPAR2 and the expression levels of these markers via the GEPIA database. The correlations between LPAR2 expression and these markers were similar to those in TIMER (**Table 3**). These findings suggested that LPAR2 was significantly related with immune infiltrating cells in HNSC and KIRC, and it seemed that LPAR2 played a significant role in HNSC and KIRC immune microenvironment.

3.8 Alterations, mutations and methylations in LPAR2 and its frequently altered neighbor

genes in patients with HNSC and KIRC.

We analyzed the LPAR2 alterations by using the cBioPortal online tool in the HNSC dataset (TCGA, Firehose Legacy) and the KIRC dataset (TCGA, Firehose Legacy). LPAR2 altered in 0.6% of 528 patients with HNSC and did not alter in 537 KIRC patients (Figure 12A, B). We also calculated mutations, methylations, mRNA expression z-scores (RNA Seq V2 RSEM), protein expression Z-scores (RPPA) and putative copy-number alterations (CNA) from GISTIC of LPAR2 in HNSC (Figure 12 A). We then constructed the 10 most frequently altered neighbor genes for LPAR2 in HNSC (Figure 12 C). The results showed that LPAR2 alterations in HNSC were strongly associated with the mutation genes, including TP53, PVALB, PNKP, LRIT3, ANXA4, EGLN2, SERTAD2, FANCI, UBASH3B, ZNF253(Figure 12 C).

4 DISCUSSION

LPA are growth-factor-like phospholipids, widely exists in human tissues and fluids^[21]. LPA participate in many biological behaviors, such as migration, proliferation, inflammation, angiogenesis, survival, and many more^[29]. LPA has several G-protein coupled receptors, named as lysophosphatidic acid receptors (LPARs)^[5, 8]. LPAR2 belongs to the endothelial differentiation gene family and contains 351 amino acids^[21, 30]. LPAR2 is unique at the C-terminus, in the proximal region it contains several putative palmitoylated cysteine residues and a dileucine motif^[31]. A few studies have suggested that LPA2 is associated with several cancers , such as breast cancer^[18, 32, 33], colon cancer^[19], ovarian cancer^[34] and stomach cancer^[35]. These studies indicate LPAR2 expression is important in cancer biology, it maybe promote gene transcription and cell proliferation in tumor microenvironment^[35].But how LPAR2 acts in tumor microenvironment is not clear at this point.

After the traditional treatment of cancer, cancer immunotherapy has become an important therapy due to its good efficacy and few side-effects. But immunotherapy has not been well studied or applied

in HNSC and KIRC. Since immunotherapy mainly targets tumor immune microenvironment, in this study, we explored the effects of LPAR2 on tumor microenvironment in HNSC and KIRC.

In this study, we examined the mRNA and protein expression levels of LPAR2 in pan tumors and corresponding normal tissues using datasets from Oncomine, TCGA in TIMER, UALCAN and HPA databases. The expression levels of LPAR2 were differentially between tumor tissues and normal tissues in multiple cancer types (Figures 1–3,6) (Table 1). Differences in data collection methods and analytical approaches may be attributed to the heterogeneity of LPAR2 expression among cancer types and databases. Nevertheless, we consistently observed higher expression of LPAR2 in HNSC and KIRC across these databases. LPAR2 seemed like oncogene in HNSC and KIRC.

Then, we employed the KM plotter, GEPIA, HPV and TCGA databases to explore the critical role of LPAR2 in patient outcomes in multiple cancer types. We found that high LPAR2 expression was significantly correlated to a poorer prognosis in KIRC (Figure 4, 5,7). But interestingly, we found that the high expression level of LPAR2 was strong associated with improved prognosis in HNSC (Figure 4, 5,7). These two different results illustrated that LPAR2 playing the opposite role in HNSC and KIRC. These findings suggest that LPAR2 is tumor suppressor gene in HNSC, and oncogene in KIRC.

Since LPAR2 expression playing significantly different prognostic roles in HNSC and KIRC, we employed UALCAN and Kaplan-Meier plotter databases to examine expression levels of LPARs with different clinical and molecular criteria and the relationship between the LPAR2 mRNA expression and prognosis in different clinical characteristics HNSC and KIRC patients. These findings suggested high LPAR2 expression was associated with advanced tumor, high tumor grade and lymph node metastasis in KIRC patients. Interestingly, by K-M plotter, we found that high LPAR2 expression levels could improve HNSC patient's prognosis only in advanced tumor stage and high grade status, but had no significant difference in KIRC patients. And high LPAR2 expression

brought good prognosis which seems to be related to its mutational burden status in HNSC patients.

For the high expression of LPAR2 affects the prognosis related to clinical characteristics in HNSC and KIRC, we used the TIMER database to explore the relationship between LPAR2 expression and the degree of immune cell infiltration. By Another important aspect of this research was the finding that the expression of LPAR2 is significantly associated with diverse immune cell infiltration levels in HNSC and KIRC. We detected a strong positive association between the expression level of LPAR2 and infiltration level of tumor purity, B cells, CD4+T cells in HNSC (Figure 12A). In KIRC, the expression levels of LPAR2 had significantly relationship with tumor purity, B cells, CD4+ T cells, neutrophils and dendritic cells levels (Figure12A). These results indicate that LPAR2 plays an Pivotal role in the regulation of immune cell infiltration in HNSC and KIRC, with particularly strong effects on tumor purity, B cells, CD4+ T cells, neutrophils and dendritic cells infiltration.

Furthermore, to investigate the role of LPAR2 in the regulation of tumor immunology in HNSC and KIRC, we analyzed the relationships between LPAR2 expression and marker genes of immune cells. Our results suggested that significant positive associations between LPAR2 expression and B cells (CD19, CD79A), M1 macrophage markers (INOS, IRF5), neutrophils markers (CD11b), Th2 markers (STAT6, STAT5A), Tfh markers (BCL6), T cell exhaustion markers (CTLA4) in HNSC ($P < 0.01$, Table 2). Remarkably, LPAR2 expression had closely relationship with INOS of M1 macrophage, STAT5A of Th2 and BCL6 of Tfh ($P < 0.0001$, $Cor > 0.2$, Figure 14A). These results indicate LPAR2 may promote the polarization of macrophages to M1 macrophages and regulate T cell responses. BCL6 recognizes DNA target sequences similar to those recognized by STAT5^[36]. Some research found that STAT5A inhibits breast cancer cell invasion and metastasis^[37]. LPAR2 may play role in HNSC by interacting STAT5A and BCL6 via prolactin-Jak2-Stat5a signal network^[36]. It deserves further study. In KIRC, LPAR2 expression levels had significantly associations with most

immune markers. Remarkably, expression of LPAR2 had closely relationship with CD3D, CD3E, of total T cells, CD19, CD79A of B cells, IRF5 of M1 macrophage, STAT5A of Th2 markers, FOXP3, CCR8 of Treg markers, PD-1, CTLA4, LAG3 of T cell exhaustion markers in KIRC ($P < 0.0001$, $Cor > 0.3$, Table 2). Our results indicated that LPAR2 activate Tregs, B cells and induce T cell exhaustion, also promote Treg responses to suppress T cell-mediated immunity. LPAR2 may therefore regulate T cell responses in KIRC. In the other hand, LPAR2 may promote the polarization of macrophages to M1 macrophages by IRF5. Together, Our findings suggested that LPAR2 is a crucial factor for the recruitment and regulation of infiltrating immune cells in HNSC and KIRC.

CONCLUSION

High LPAR2 expression playing significantly different prognostic roles in HNSC and KIRC, might be due to associate with different immune markers. And LPAR2 is a pivotal player in governing immune cell infiltration and a valuable prognostic biomarker to guide treatment in patients with HNSC and KIRC.

DECLARATIONS

List of 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; 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; KIRP: kidney renal papillary cell carcinoma; LIHC: liver hepatocellular carcinoma; ESCA: Esophageal carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; PRAD: prostate adenocarcinoma; READ: Rectum adenocarcinoma; UCEC: uterine corpus endometrial carcinoma; CHOL: cholangial carcinoma; COAD: colon adenocarcinoma; CECS: Cervical squamous cell carcinoma and endocervical adenocarcinoma; STAD: stomach adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; THCA: Thyroid carcinoma; GBM: Glioblastoma multiforme; PAAD: Pancreatic adenocarcinoma; THYM: Thymoma; ACC: Adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; PCPG: Pheochromocytoma and Paraganglioma; UCEC: uterine corpus endometrial carcinoma; UCS: Uterine Carcinosarcoma; OS: overall survival; DFS: disease-free survival; RFS: relapse-free survival; PPS: post-progression survival; DSS: disease-specific survival; DMFS: distant metastasis-free survival; FP: first progression; TAM: Tumor-associated macrophages; NK cells: natural killer cells;

DCs: dendritic cells; Tfh: follicular helper T cell; Th: T helper cell.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors are consent for the publication of this work.

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.

Competing interests

The authors declare that they have no competing interests.

Funding statement

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Author 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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References

- [1] Liu S, Umezu-Goto M, Murph M, et al. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. *Cancer Cell*. 2009. 15(6): 539-50.
- [2] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012. 490(7418): 61-70.
- [3] Hartman ZC, Poage GM, den Hollander P, et al. Growth of triple-negative breast cancer cells relies upon coordinate autocrine expression of the proinflammatory cytokines IL-6 and IL-8. *Cancer Res*. 2013. 73(11): 3470-80.
- [4] Chrencik JE, Roth CB, Terakado M, et al. Crystal Structure of Antagonist Bound Human Lysophosphatidic Acid Receptor 1. *Cell*. 2015. 161(7): 1633-43.
- [5] Tager AM, LaCamera P, Shea BS, et al. The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nat Med*. 2008. 14(1): 45-54.
- [6] Farquhar MJ, Humphreys IS, Rudge SA, et al. Autotaxin-lysophosphatidic acid receptor signalling regulates hepatitis C virus replication. *J Hepatol*. 2017. 66(5): 919-929.
- [7] Orosa B, García S, Martínez P, González A, Gómez-Reino JJ, Conde C. Lysophosphatidic acid receptor inhibition as a new multipronged treatment for rheumatoid arthritis. *Ann Rheum Dis*. 2014. 73(1): 298-305.
- [8] Llona-Minguez S, Ghassemian A, Helleday T. Lysophosphatidic acid receptor (LPA1) modulators: The current pharmacological toolbox. *Prog Lipid Res*. 2015. 58: 51-75.
- [9] Marshall JC, Collins JW, Nakayama J, et al. Effect of inhibition of the lysophosphatidic acid receptor 1 on metastasis and metastatic dormancy in breast cancer. *J Natl Cancer Inst*. 2012. 104(17): 1306-19.
- [10] Hama K, Aoki J. LPA(3), a unique G protein-coupled receptor for lysophosphatidic acid. *Prog Lipid Res*. 2010. 49(4): 335-42.
- [11] Mansell JP, Barbour M, Moore C, et al. The synergistic effects of lysophosphatidic acid receptor agonists and calcitriol on MG63 osteoblast maturation at titanium and hydroxyapatite surfaces. *Biomaterials*. 2010. 31(2): 199-206.
- [12] Mazzocca A, Dituri F, De Santis F, et al. Lysophosphatidic acid receptor LPA6 supports the tumorigenicity of hepatocellular carcinoma. *Cancer Res*. 2015. 75(3): 532-43.
- [13] Zhang H, Xu X, Gajewiak J, et al. Dual activity lysophosphatidic acid receptor pan-antagonist/autotaxin inhibitor reduces breast cancer cell migration in vitro and causes tumor regression in vivo. *Cancer Res*. 2009. 69(13): 5441-9.
- [14] Allanore Y, Distler O, Jagerschmidt A, et al. Lysophosphatidic Acid Receptor 1 Antagonist SAR100842 for Patients With Diffuse Cutaneous Systemic Sclerosis: A Double-Blind, Randomized, Eight-Week Placebo-Controlled Study Followed by a Sixteen-Week Open-Label Extension Study. *Arthritis Rheumatol*. 2018. 70(10): 1634-1643.
- [15] Park SY, Jeong KJ, Panupinthu N, et al. Lysophosphatidic acid augments human hepatocellular carcinoma cell invasion through LPA1 receptor and MMP-9 expression. *Oncogene*. 2011. 30(11): 1351-

- [16] Zuo C, Li X, Huang J, et al. Osteoglycin attenuates cardiac fibrosis by suppressing cardiac myofibroblast proliferation and migration through antagonizing lysophosphatidic acid 3/matrix metalloproteinase 2/epidermal growth factor receptor signalling. *Cardiovasc Res*. 2018. 114(5): 703-712.
- [17] Noguchi K, Ishii S, Shimizu T. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. *J Biol Chem*. 2003. 278(28): 25600-6.
- [18] Sun K, Cai H, Duan X, et al. Aberrant expression and potential therapeutic target of lysophosphatidic acid receptor 3 in triple-negative breast cancers. *Clin Exp Med*. 2015. 15(3): 371-80.
- [19] Shukla PK, Meena AS, Gangwar R, et al. LPAR2 receptor activation attenuates radiation-induced disruption of apical junctional complexes and mucosal barrier dysfunction in mouse colon. *FASEB J*. 2020. 34(9): 11641-11657.
- [20] Kuriyama S, Theveneau E, Benedetto A, et al. In vivo collective cell migration requires an LPAR2-dependent increase in tissue fluidity. *J Cell Biol*. 2014. 206(1): 113-27.
- [21] Deng W, Shuyu E, Tsukahara R, et al. The lysophosphatidic acid type 2 receptor is required for protection against radiation-induced intestinal injury. *Gastroenterology*. 2007. 132(5): 1834-51.
- [22] Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res*. 2017. 77(21): e108-e110.
- [23] Chandrashekar DS, Bashel B, Balasubramanya S, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*. 2017. 19(8): 649-658.
- [24] Nagy Á, Munkácsy G, Györffy B. Pancancer survival analysis of cancer hallmark genes. *Sci Rep*. 2021. 11(1): 6047.
- [25] Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics. Tissue-based map of the human proteome. *Science*. 2015. 347(6220): 1260419.
- [26] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013. 6(269): p11.
- [27] Azimi F, Scolyer RA, Rumcheva P, 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(21): 2678-83.
- [28] Ohtani H. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. *Cancer Immun*. 2007. 7: 4.
- [29] Taniguchi R, Inoue A, Sayama M, et al. Structural insights into ligand recognition by the lysophosphatidic acid receptor LPA6. *Nature*. 2017. 548(7667): 356-360.
- [30] Lin S, Wang D, Iyer S, et al. The absence of LPA2 attenuates tumor formation in an experimental model of colitis-associated cancer. *Gastroenterology*. 2009. 136(5): 1711-20.
- [31] Lee SJ, Ritter SL, Zhang H, Shim H, Hall RA, Yun CC. MAGI-3 competes with NHERF-2 to negatively regulate LPA2 receptor signaling in colon cancer cells. *Gastroenterology*. 2011. 140(3): 924-34.
- [32] Kitayama J, Shida D, Sako A, et al. Over-expression of lysophosphatidic acid receptor-2 in human invasive ductal carcinoma. *Breast Cancer Res*. 2004. 6(6): R640-6.
- [33] Sun K, Duan X, Cai H, et al. Curcumin inhibits LPA-induced invasion by attenuating RhoA/ROCK/MMPs pathway in MCF7 breast cancer cells. *Clin Exp Med*. 2016. 16(1): 37-47.
- [34] Kowalczyk-Zieba I, Woclawek-Potocka I, Wasniewski T, et al. LPAR2 and LPAR4 are the Main Receptors Responsible for LPA Actions in Ovarian Endometriotic Cysts. *Reprod Sci*. 2019. 26(1): 139-

- [35] Ren Z, Zhang C, Ma L, et al. Lysophosphatidic acid induces the migration and invasion of SGC-7901 gastric cancer cells through the LPA2 and Notch signaling pathways. *Int J Mol Med*. 2019. 44(1): 67-78.
- [36] Tran TH, Utama FE, Lin J, et al. Prolactin inhibits BCL6 expression in breast cancer through a Stat5a-dependent mechanism. *Cancer Res*. 2010. 70(4): 1711-21.
- [37] Tran TH, Utama FE, Sato T, et al. Loss of Nuclear Localized Parathyroid Hormone-Related Protein in Primary Breast Cancer Predicts Poor Clinical Outcome and Correlates with Suppressed Stat5 Signaling. *Clin Cancer Res*. 2018. 24(24): 6355-6366.

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

Cancer	Cancer type	<i>P</i> -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. Normal	5.43E-11	2.342	3%	367	Sanchez-Carbayo Bladder 2
	Superficial Bladder Cancer vs. Normal	1.35E-7	1.582	3%	375	Dyrskjot Bladder 3
	Superficial Bladder Cancer vs. Normal	8.31E-6	1.504	4%	652	Lee Bladder
Brain and CNS Cancer	Anaplastic Astrocytoma vs. Normal	4.10E-5	2.255	8%	1521	Sun Brain
Breast cancer	Mixed Lobular and Ductal Breast Carcinoma vs. Normal	3.13E-9	1.889	1%	50	TCGA Breast
	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 caner	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. Normal	6.08E-12	-1.797	2%	314	Piccaluga Lymphoma
Other cancer Sarcoma	Testicular Seminoma vs. Normal	7.73E-8	1.859	3%	284	Sperger Others
	Gastrointestinal Stromal Tumor vs. Normal	3.90E-10	-4.256	2%	269	Cho Gastric

Table 2 Correlation analysis between LPAR2 and relate genes and markers of immune cells in TIMER

Description	Gene markers	HNSC				KIRC			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
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	***
	CD115(CSF1R)	0.024	5.91e-01	0.086	5.52e-02	0.289	***	0.266	***
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	***
	IL10	-0.063	1.50e-01	-0.002	9.66e-01	0.118	*	0.072	1.20e-01
M1Macrophage	INOS(NOS2)	0.262	***	0.252	***	-0.127	*	-0.127	*
	IRF5	0.173	***	0.18	***	0.301	***	0.301	***
	COX2(PTGS2)	-0.017	6.95e-01	-0.043	3.38e-01	0.057	1.92e-01	0.057	1.92e-01

M2 Macrophage	CD163	-0.017	6.92e-01	0.04	3.76e-01	0.123	*	0.113	1.53e-02
	VSIG4	-0.027	5.33e-01	0.034	4.52e-01	0.247	***	0.233	***
	MS4A4A	0.006	8.91e-01	0.069	1.28e-01	0.12	*	0.081	8.11e-02
Neutrophils	CD66b(CEACAM8)	0.053	2.29e-01	0.045	3.22e-01	-0.007	8.72e-01	-0.012	7.90e-01
	CD11b(ITGAM)	0.131	*	0.161	**	0.271	***	0.261	***
	CCR7	0.083	5.91e-02	0.154	**	0.275	***	0.25	***
Naturalkiller 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
	KIR2DL4	0.009	8.44e-01	0.063	1.62e-01	0.136	*	0.122	*
	KIR3DL1	0.012	7.83e-01	0.042	3.57e-01	-0.066	1.29e-01	-0.051	2.73e-01
	KIR3DL2	0.036	4.01e-01	0.069	1.26e-01	0.059	1.71e-01	0.071	1.29e-01
	KIR3DL3	0.059	1.76e-01	0.094	3.78e-02	0.028	5.16e-01	0.012	8.01e-01
	KIR2DS4	0.052	2.38e-01	0.087	5.45e-02	0.004	9.36e-01	0.01	8.35e-01

(Continues)

Table 2 (Continued)

Description	Gene markers	HNSC				KIRC			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
Dendritic cell	HLA-DPB1	0.065	1.40e-01	0.135	2.64e-03	0.191	***	0.178	***
	HLA-DQB1	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
	BCDA-1(CD1C)	0.019	6.66e-01	0.084	6.20e-02	0.04	3.58e-01	0.002	9.66e-01
	BDCA-4(NRP1)	-0.067	1.25e-01	-0.026	5.62e-01	-0.123	*	-0.169	**
	CD11c(ITGAX)	0.095	3.02e-02	0.173	*	0.315	***	0.313	***
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	**
	IFN- γ (IFNG)	0.017	7.05e-01	0.076	9.04e-02	0.284	***	0.243	***
	TNF- α (TNF)	0.052	2.32e-01	0.067	1.40e-01	0.24	***	0.212	***
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	**
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(TGFB1)	-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

Table 3 Correlation analysis between LPAR2 and relate genes and immune markers in GEPIA.

Description	Gene markers	HNSC				KIRC			
		Tumor		Normal		Tumor		Normal	
		R	P	R	P	R	P	R	P
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
M1Macrophage	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(ITGAM)	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(ITGAX)	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
Tfh	BCL6	0.26	***	0.4	**	0.33	***	0.78	***
Treg	FOXP3	0.087	*	0.34	*	0.33	***	0.48	***
	CCR8	0.06	0.17	0.13	0.39	0.33	***	-0.16	0.17
	TGF β (TGFB1)	0.039	0.38	0.39	**	0.34	***	0.76	***
Tcell 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	***

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

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.

Analysis Type by Cancer	Cancer vs. Normal		Cancer vs. Cancer			
			Cancer Histology		Multi-cancer	
Bladder Cancer	4		1	1		
Brain and CNS Cancer	1		1			1
Breast Cancer	4		1		3	
Cervical Cancer						
Colorectal Cancer	1				5	
Esophageal Cancer						
Gastric Cancer						
Head and Neck Cancer						
Kidney Cancer	5	2	2	3		
Leukemia		3	2	1		
Liver Cancer						1
Lung Cancer	1	1				1
Lymphoma	1	1	1			
Melanoma			1	1		3
Myeloma						
Other Cancer	1		1	1		2
Ovarian Cancer					1	
Pancreatic Cancer						
Prostate Cancer						
Sarcoma		1	3			2
Significant Unique Analyses	18	8	13	7	9	8
Total Unique Analyses	428		725		255	



Figure 1. The transcription levels of LPAR2 in different cancers (ONCOMINE).

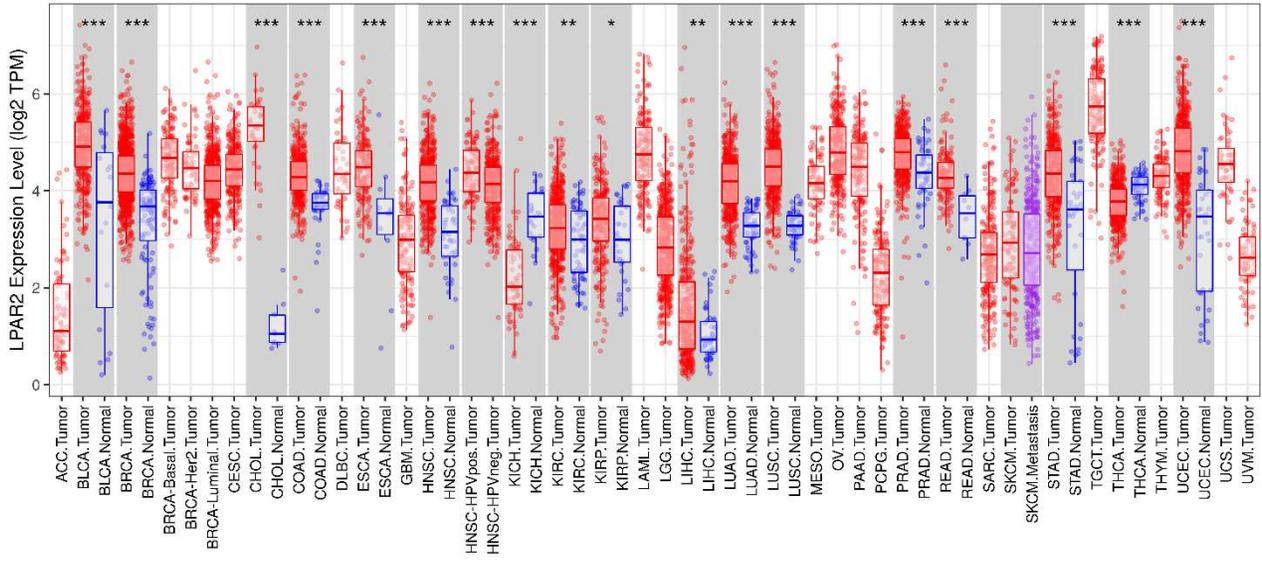


Figure 2. LPAR2 expression levels in different tumor types from TCGA database were determined by TIMER

*P < 0.05, **P < 0.01, ***P < 0.001

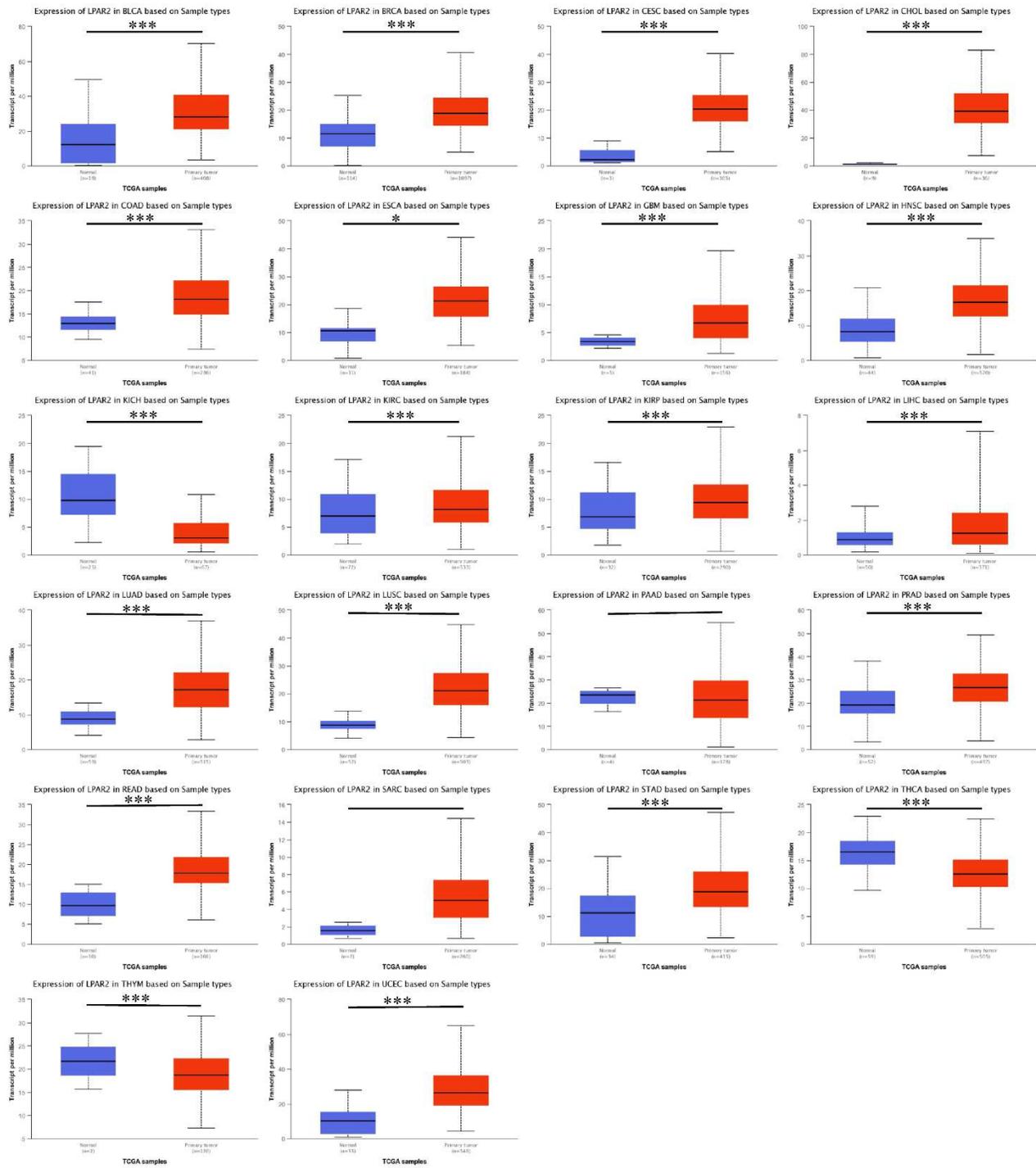


Figure 3. LPAR2 mRNA expression levels in different tumor types from TCGA database were determined by UCLAN.

*P < 0.05, **P < 0.01, ***P < 0.001

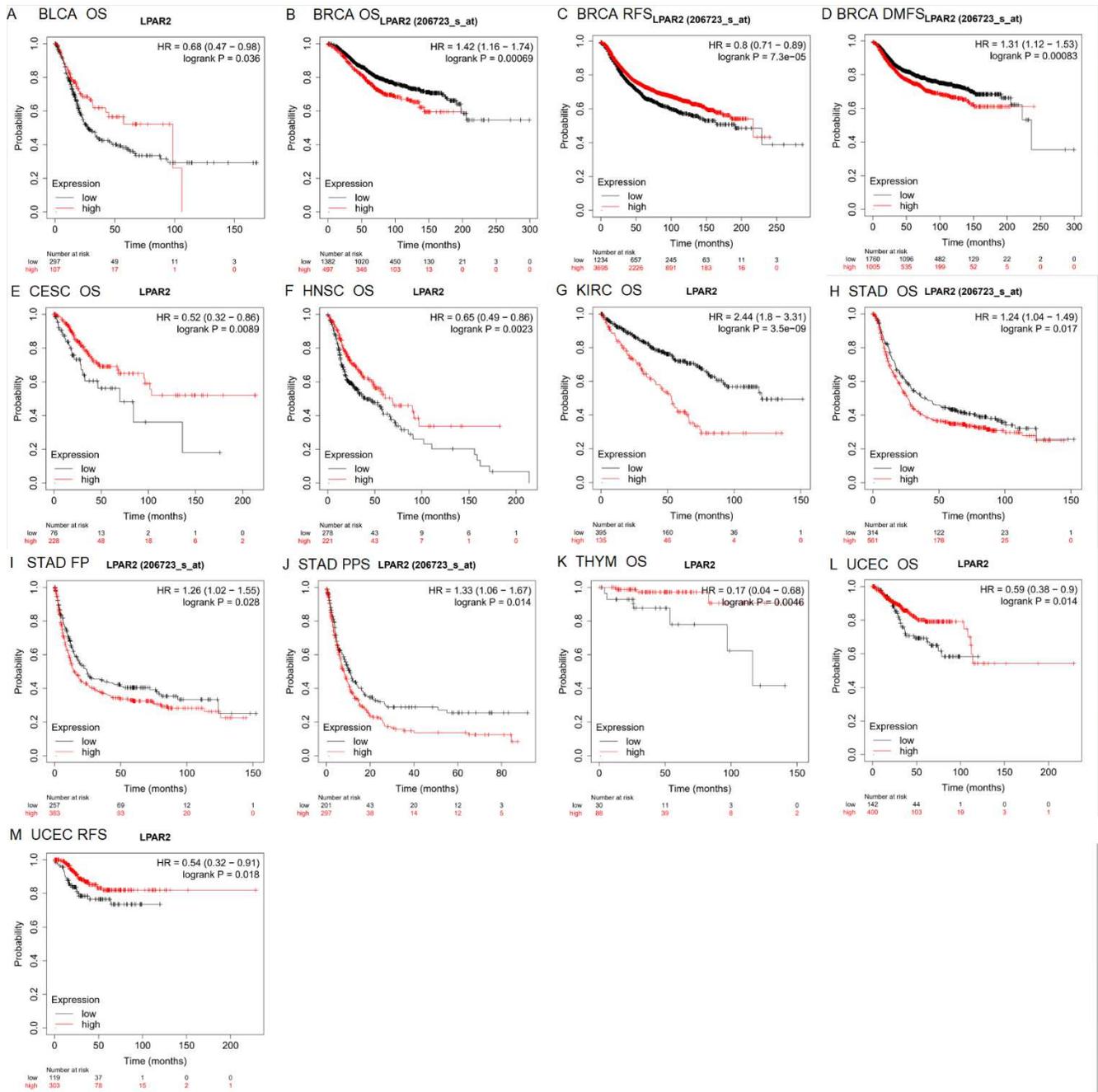


Figure 4 Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancers in the Kaplan-Meier plotter databases. (A-M) OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; DSS, disease-specific survival. DMFS, distant metastasis-free survival. FP, first progression

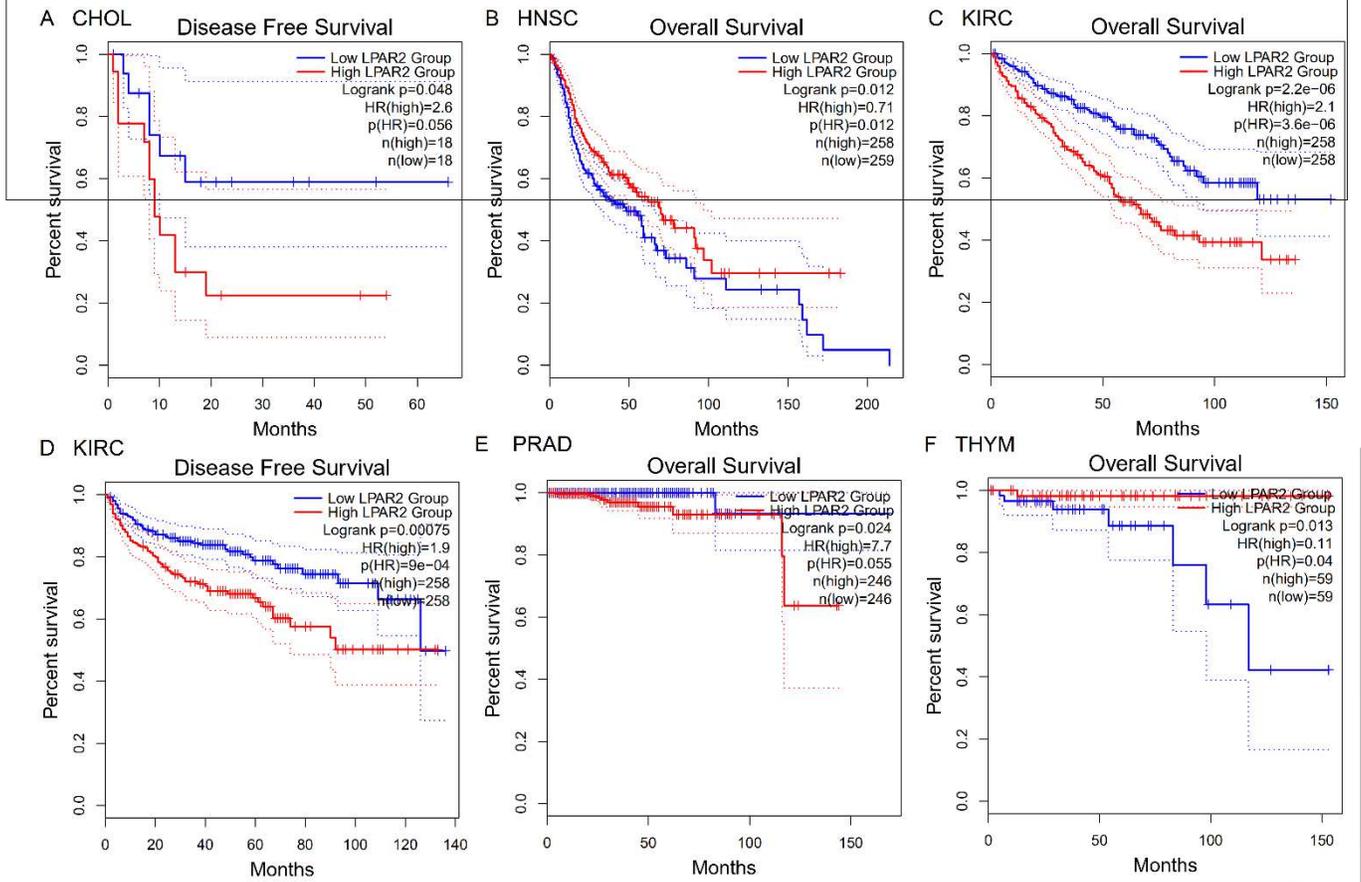


Figure 5 Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancer in GEPIA databases. (A-F)

OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; DSS, disease-specific survival. DMFS, distant metastasis-free survival.

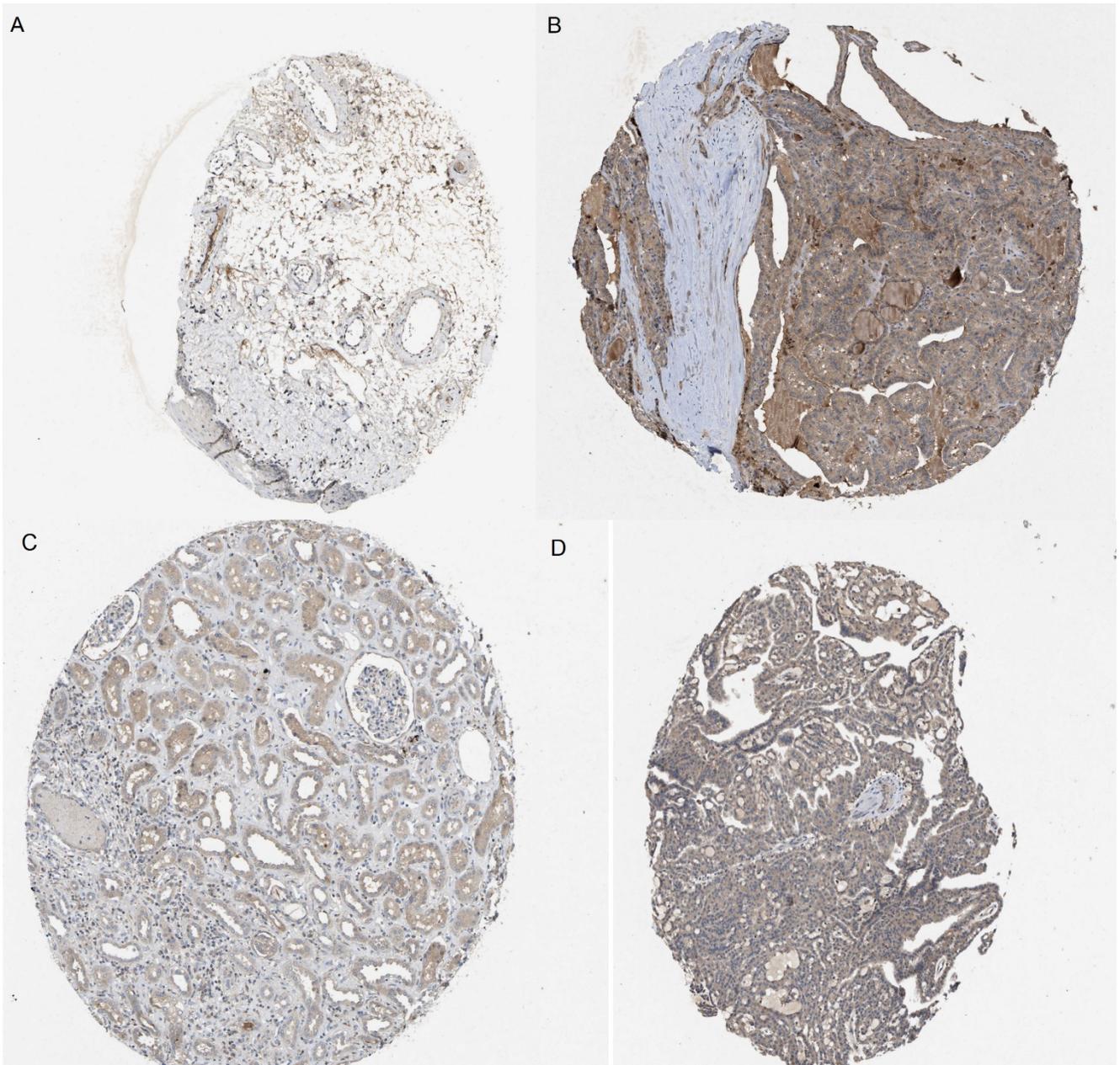


Figure 6 Representative immunohistochemistry images of different LPAR2 in HNSC and KIRC tissues and corresponding normal tissues from the human protein atlas database (HPA).

A: Oral normal tissue; B: Head-Neck Squamous cell carcinoma tissue; C: Kidney normal tissue; D: Kidney renal clear cell carcinoma tissue.

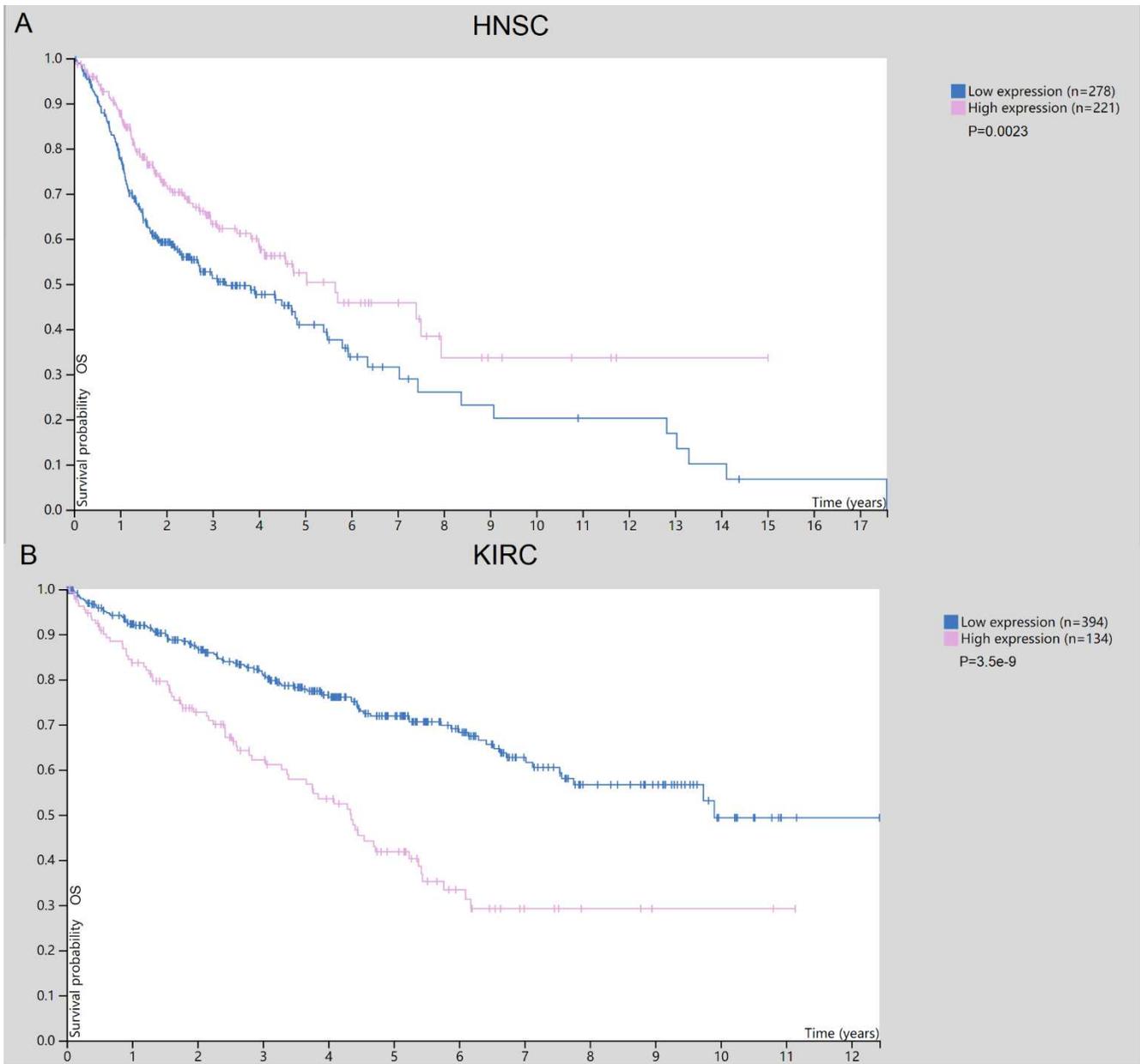


Figure 7. LPAR2 protein expression levels in HNSC and KIRC from the human protein atlas database (HPA).

A: OS OF HNSC; B: OS OF KIRC.

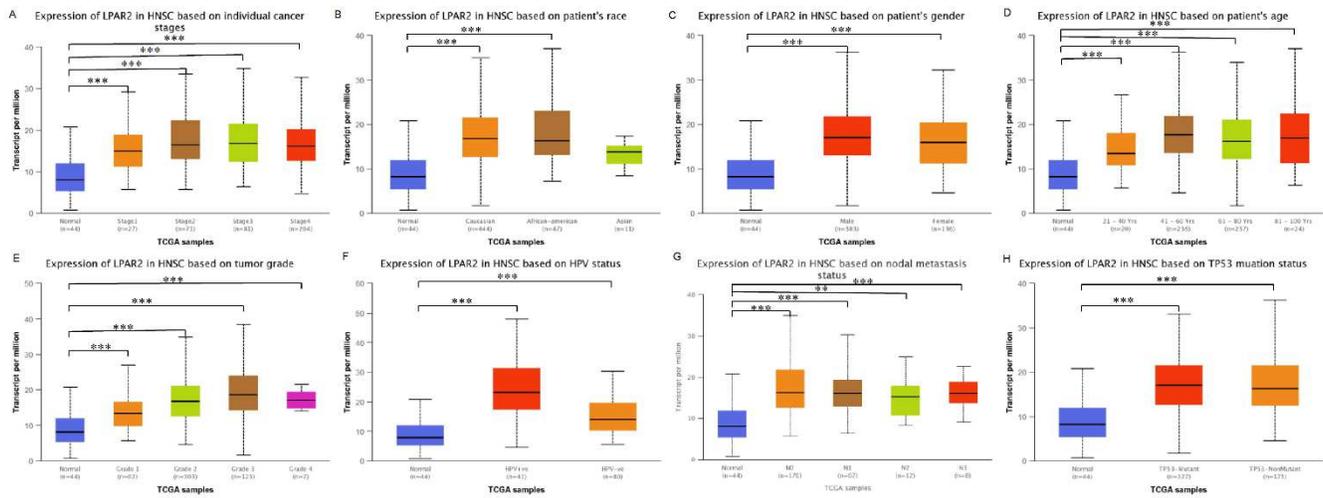


Figure 8 the relationship between the LPAR2 mRNA expression and clinical characteristics of HNSC patients from TCGA database in UCLAN.

*P < 0.05, **P < 0.01, ***P < 0.001

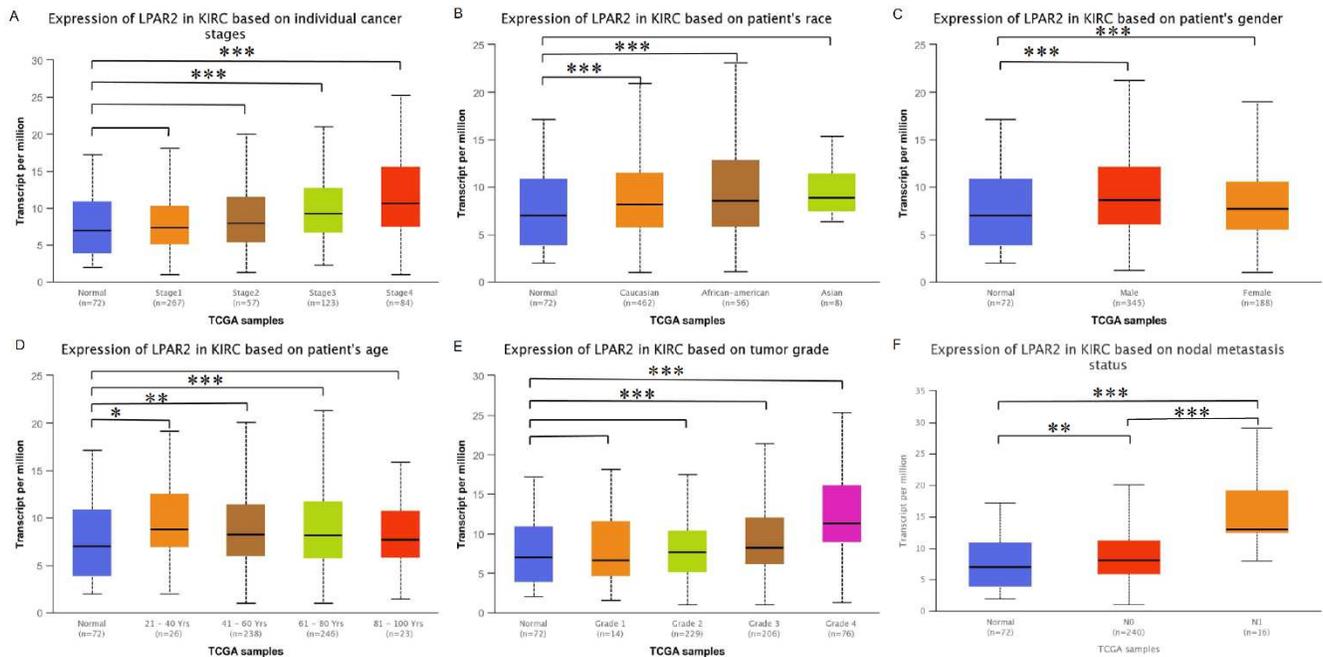


Figure 9 the relationship between the LPAR2 mRNA expression and clinical characteristics of KIRC patients from TCGA database in UCLAN.

*P < 0.05, **P < 0.01, ***P < 0.001

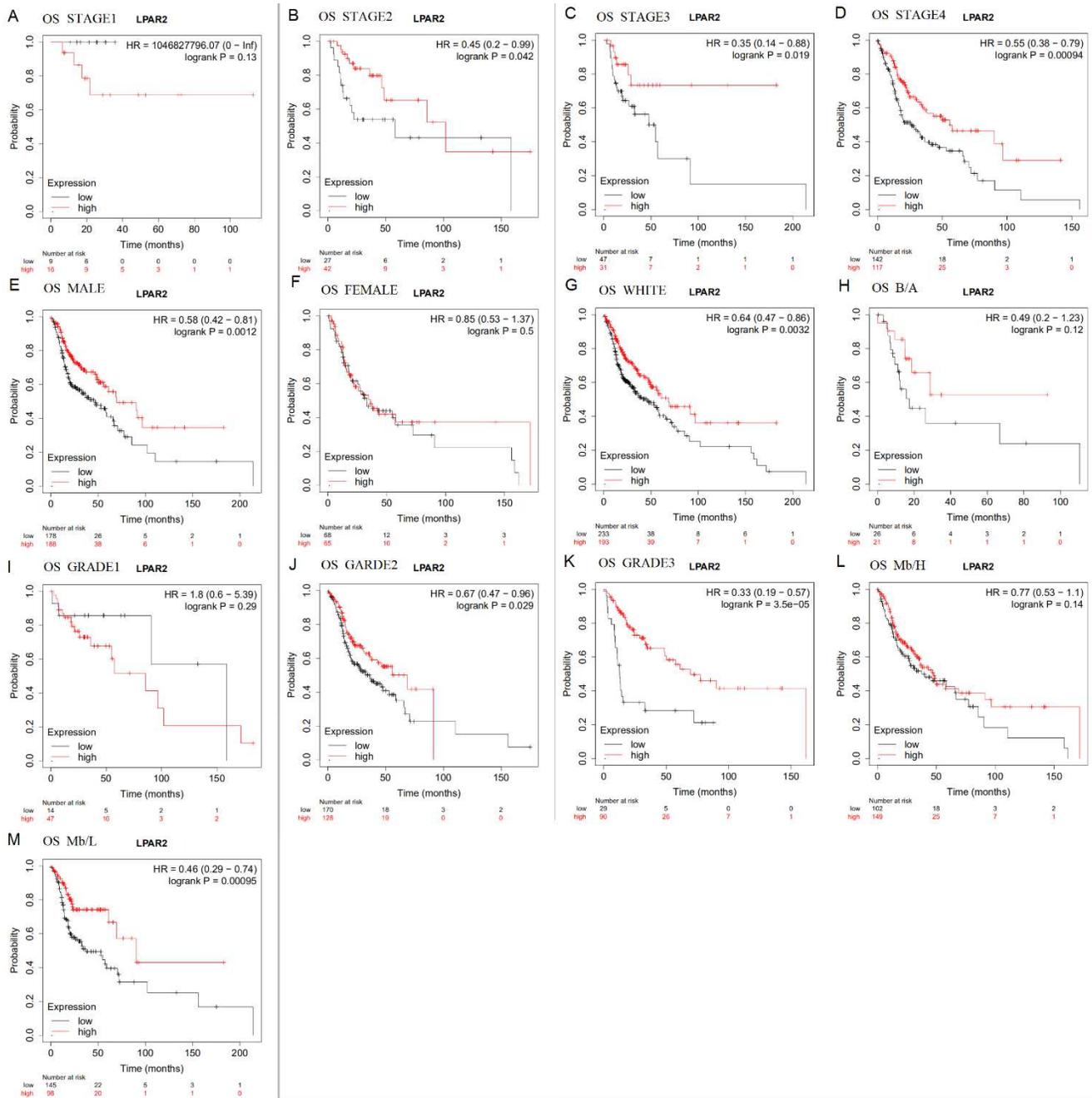


Figure 10 the relationship between the LPAR2 mRNA expression and prognosis in HNSC patients with different clinical characteristics in Kaplan-Meier plotter databases.

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

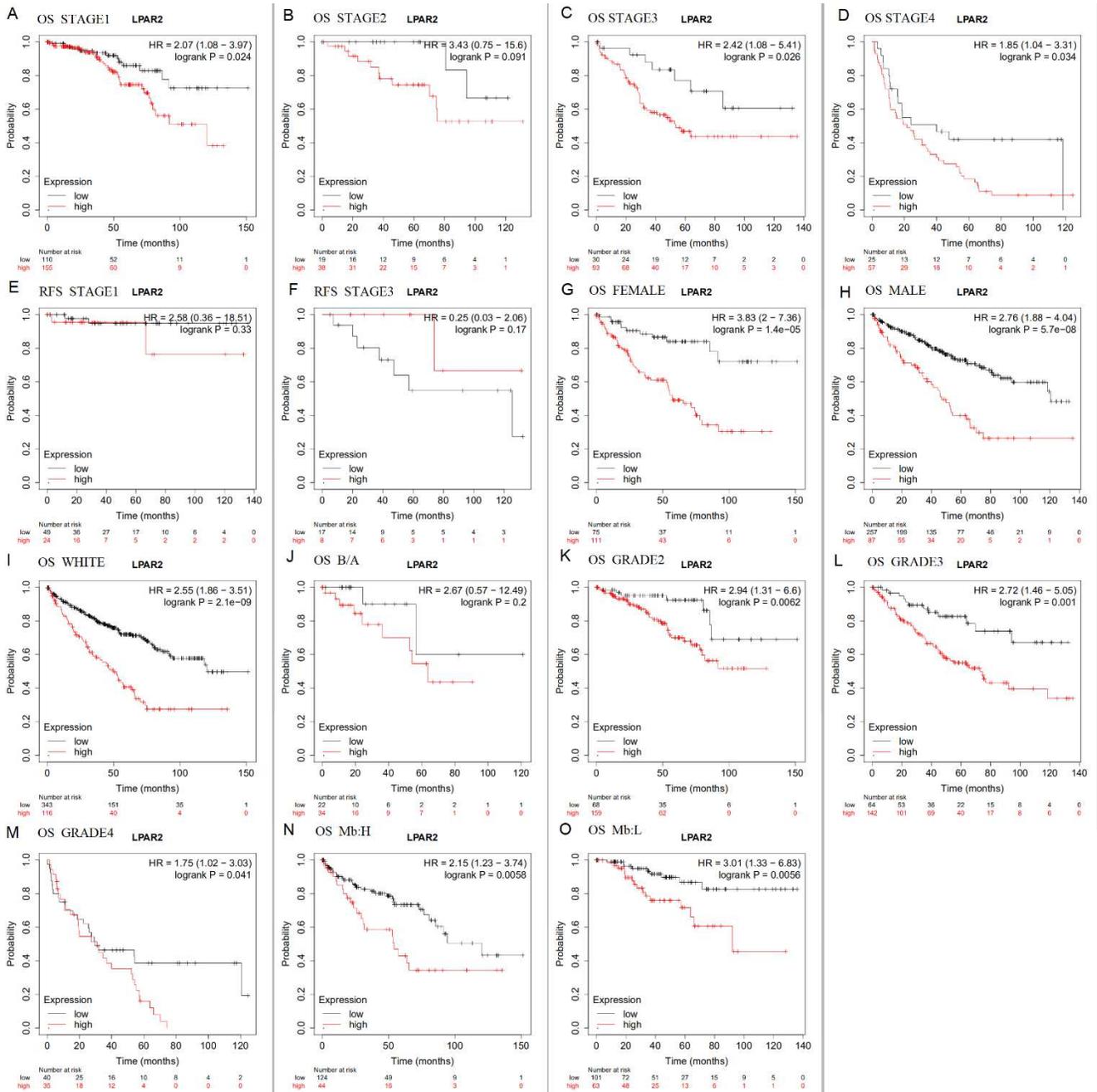


Figure 11 the relationship between the LPAR2 mRNA expression and prognosis in KIRC patients with different clinical characteristics in Kaplan-Meier plotter databases.

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

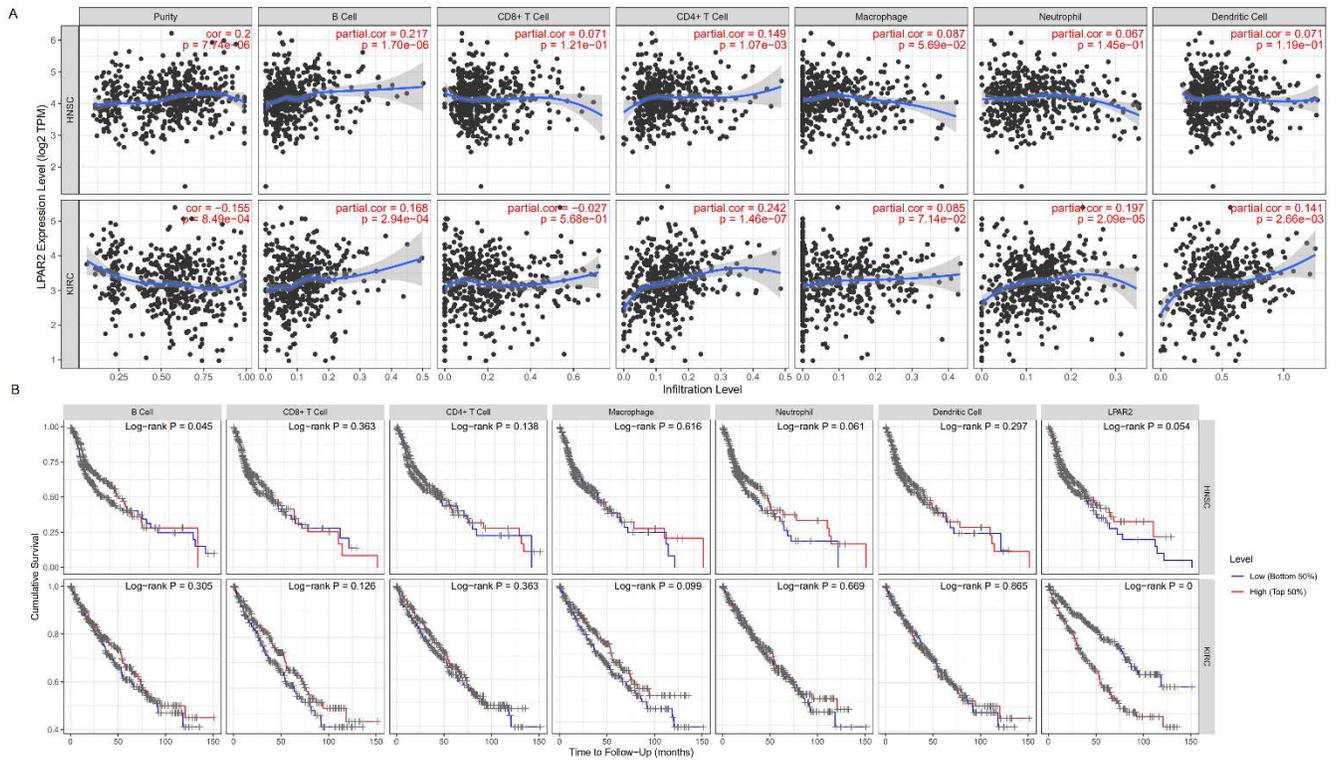


Figure 12 A) Correlation of LPAR2 expression with immune infiltration level in HNSC and KIRC.

B) Kaplan-Meier plots of immune infiltration and LPAR2 expression levels in HNSC and KIRC.

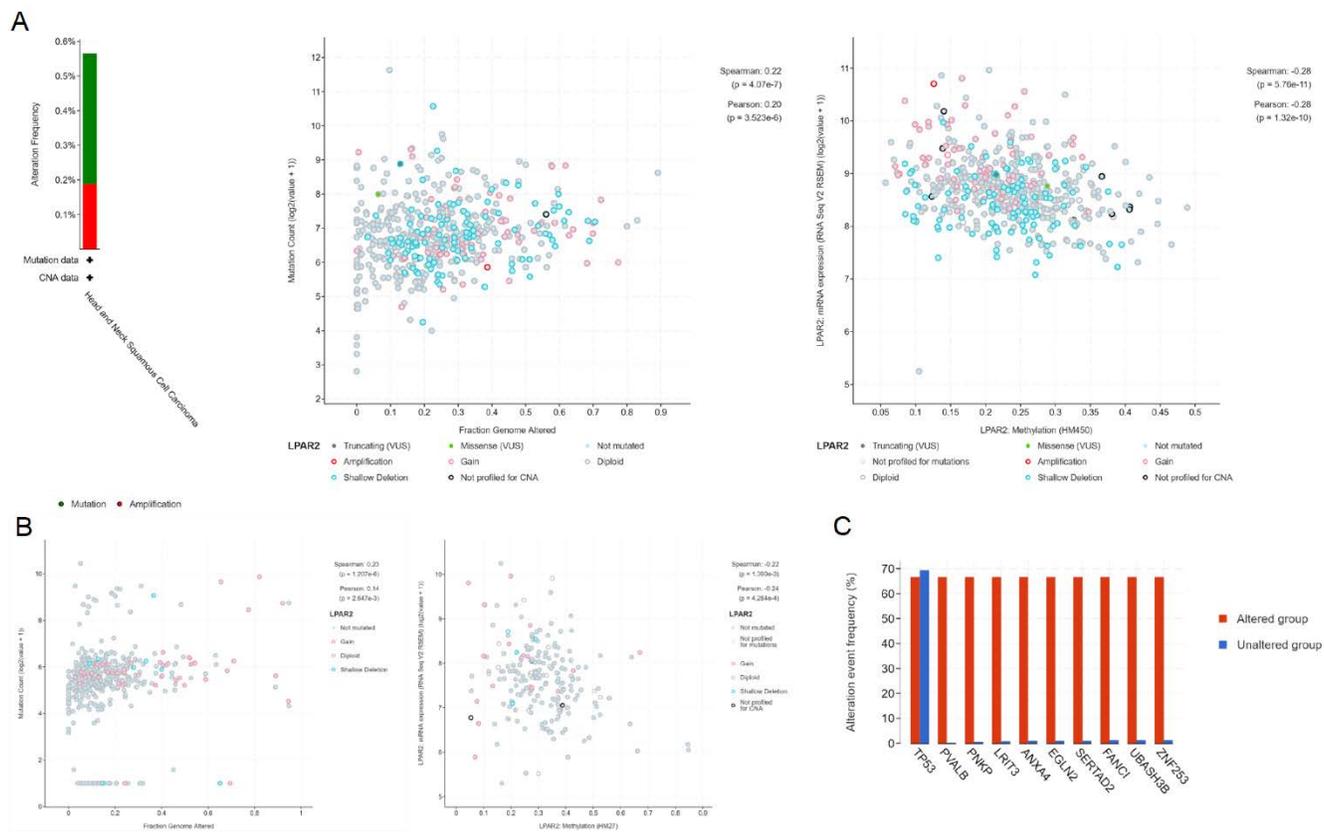


Figure 13 A) LPAR2 alterations in HNSC; B) LPAR2 did not alter in KIRC; C) the 10 most frequently altered neighbor genes for LPAR2 in HNSC (cBioPortal).

Figures

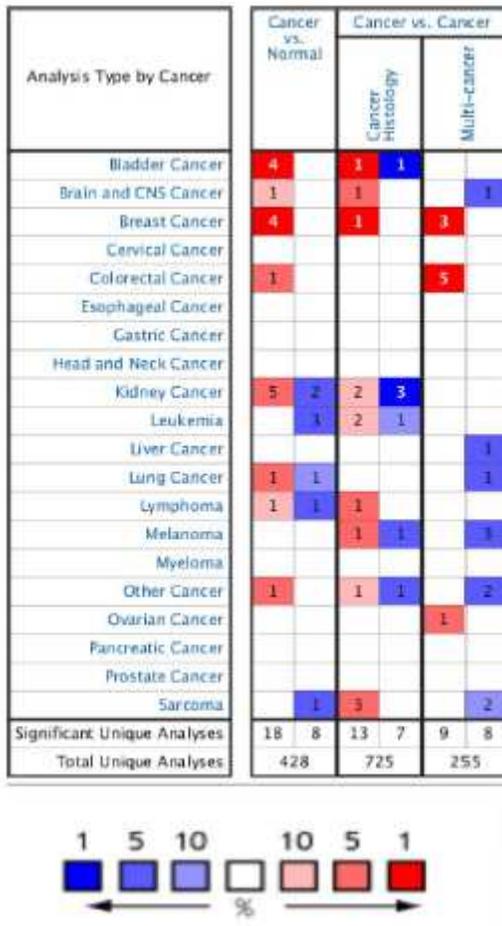


Figure 1

The transcription levels of LPAR2 in different cancers

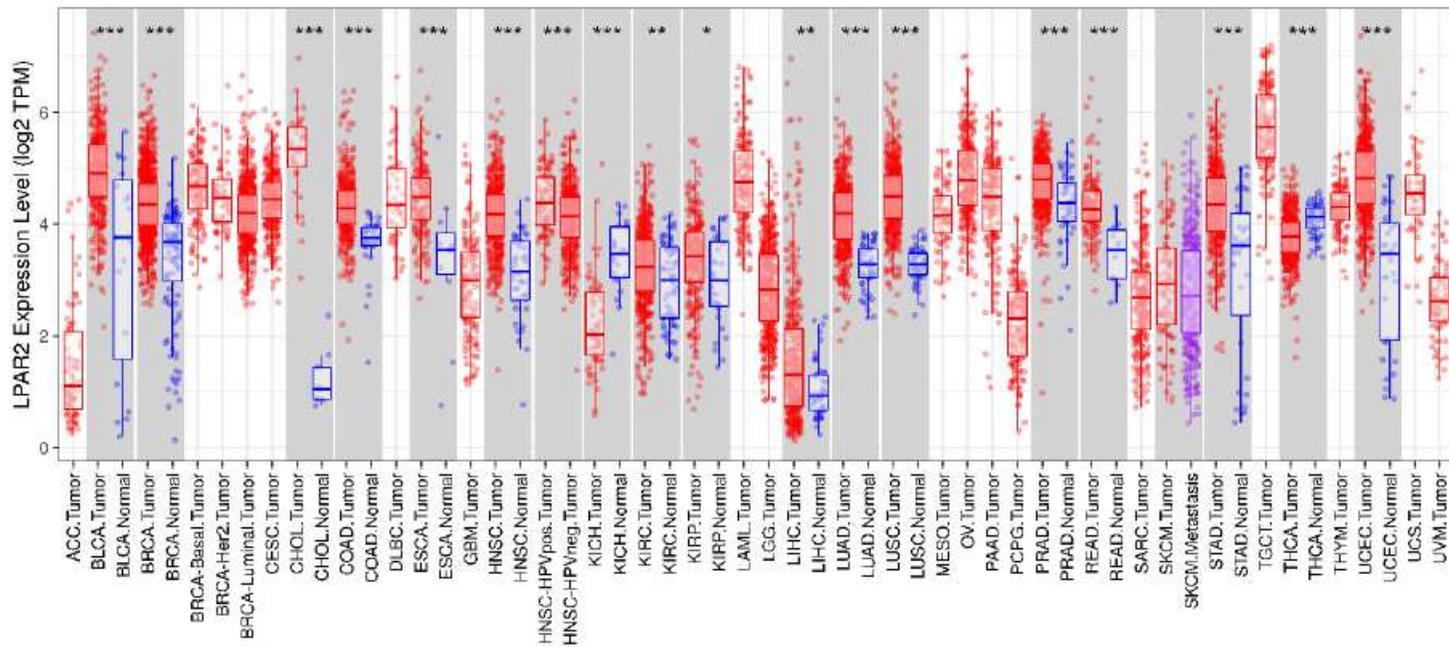


Figure 2

LPAR2 expression levels in different tumor types from TCGA database were determined by TIMER *P < 0.05, **P < 0.01, ***P < 0.001

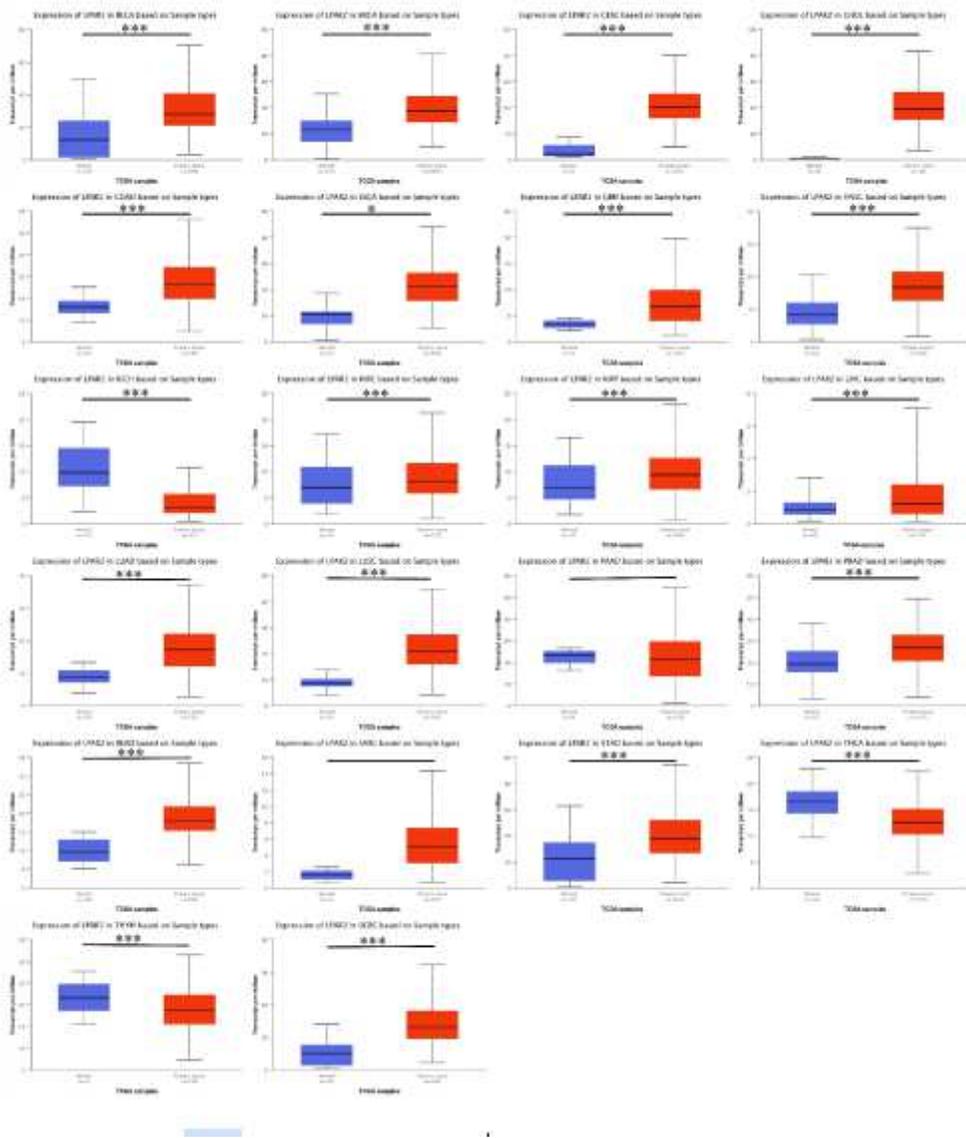


Figure 3

LPAR2 mRNA expression levels in different tumor types from TCGA database were determined by UCLAN.

*P < 0.05, **P < 0.01, ***P < 0.001

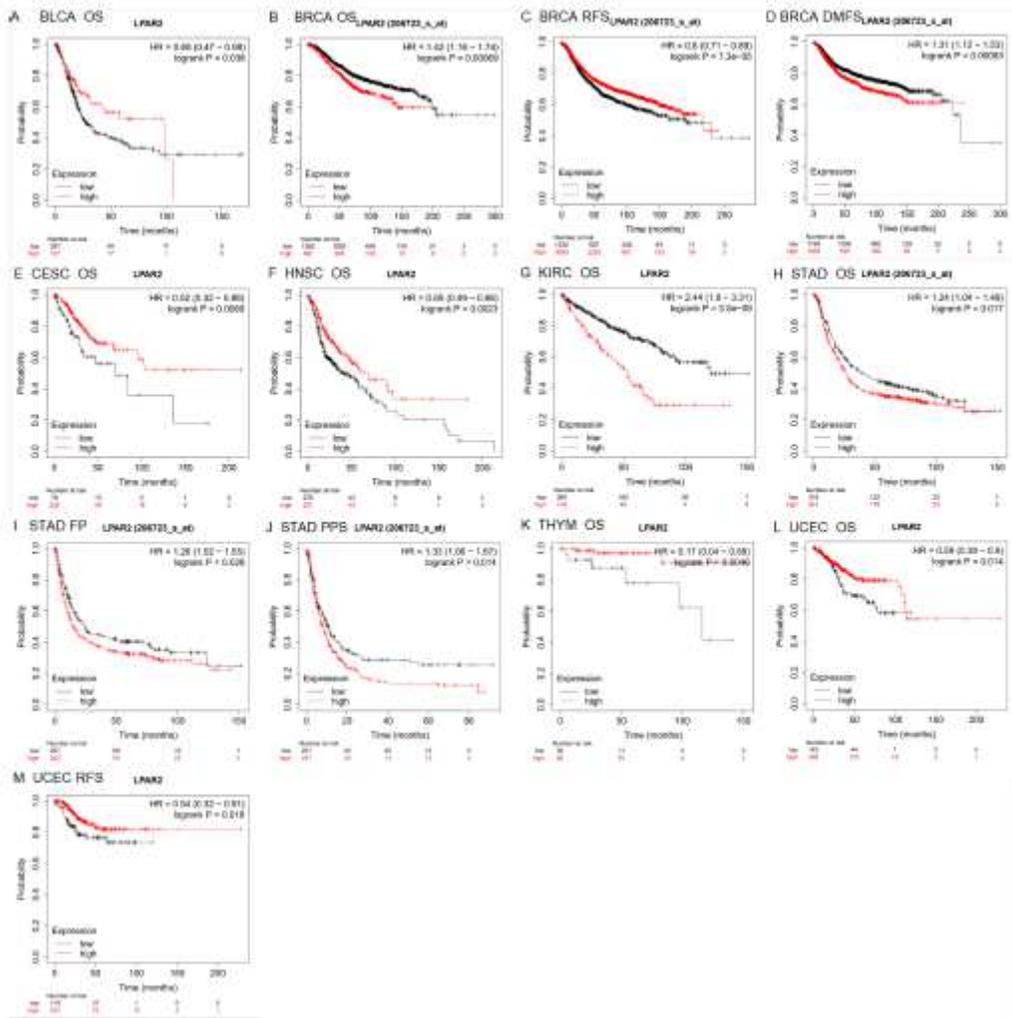


Figure 4

Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancers in the Kaplan-Meier plotter databases. (A-M) OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; DSS, disease-specific survival. DMFS, distant metastasis-free survival. FP, first progression

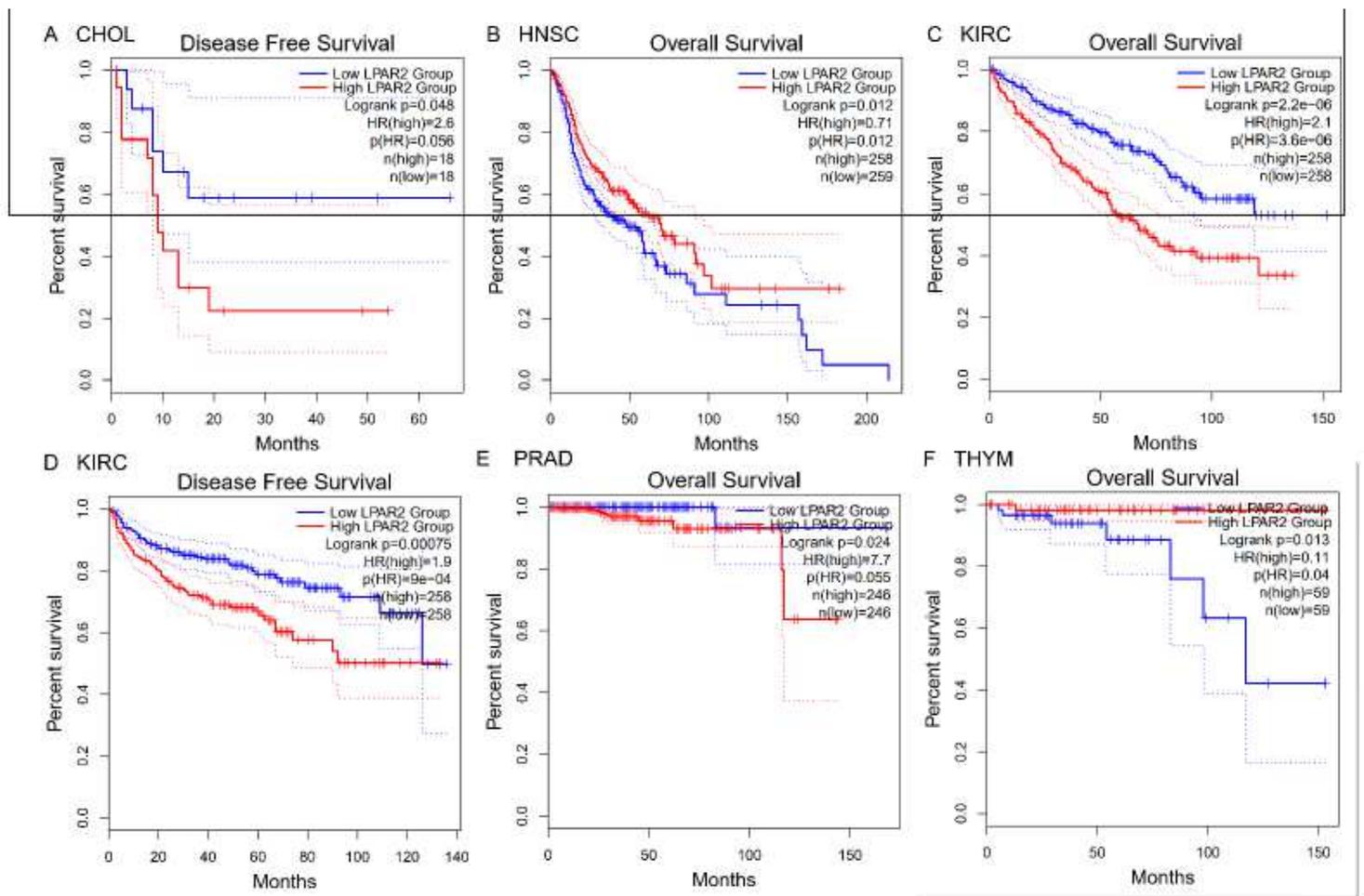


Figure 5

Kaplan-Meier survival curves comparing the high and low expression of LPAR2 in different types of cancer in GEPIA databases. (A-F) OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; DSS, disease-specific survival. DMFS, distant metastasis-free survival.

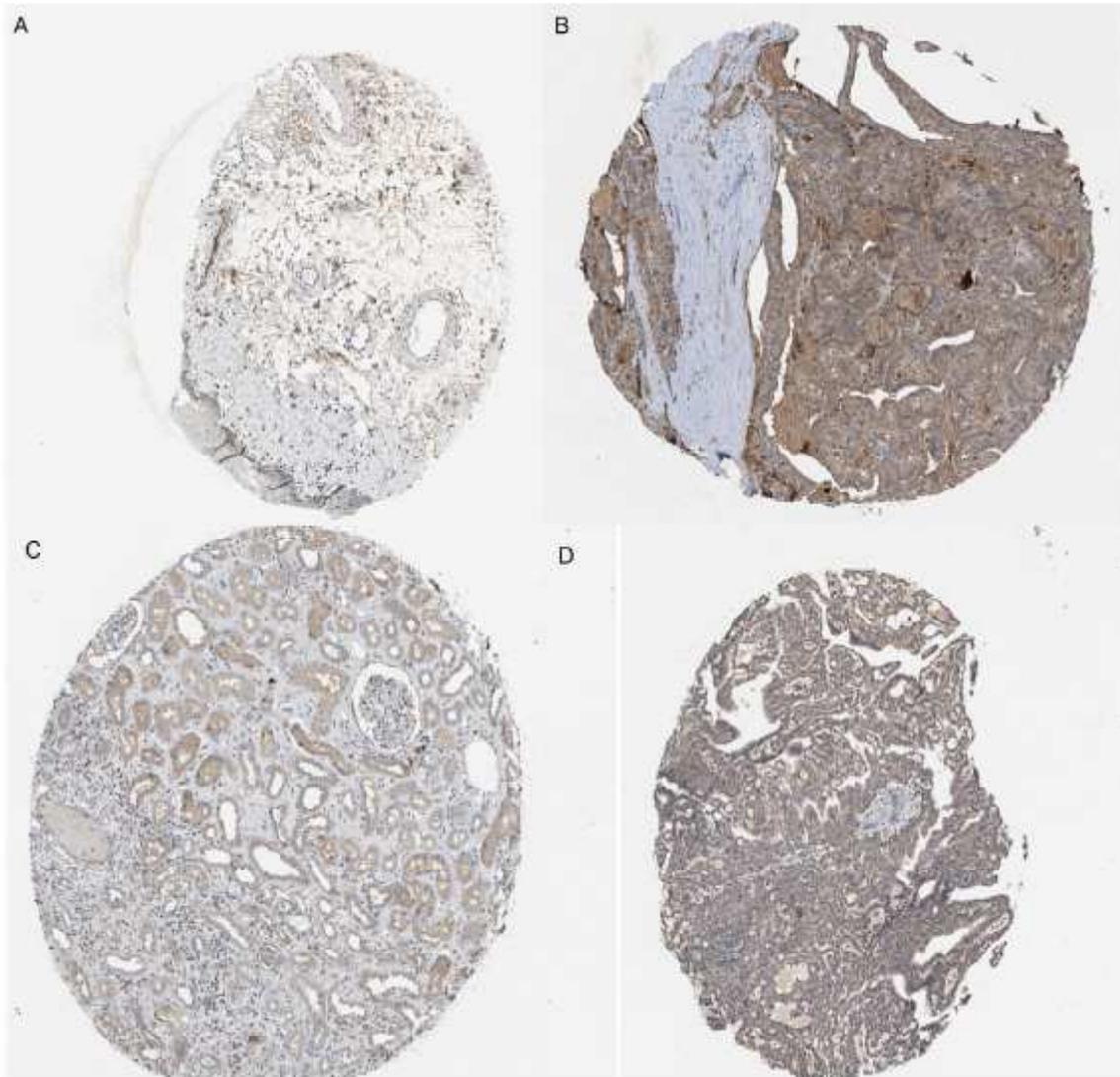


Figure 6

Representative immunohistochemistry images of different LPAR2 in HNSC and KIRC tissues and corresponding normal tissues from the human protein atlas database (HPA). A: Oral normal tissue; B: Head-Neck Squamous cell carcinoma tissue; C: Kidney normal tissue; D: Kidney renal clear cell carcinoma tissue.

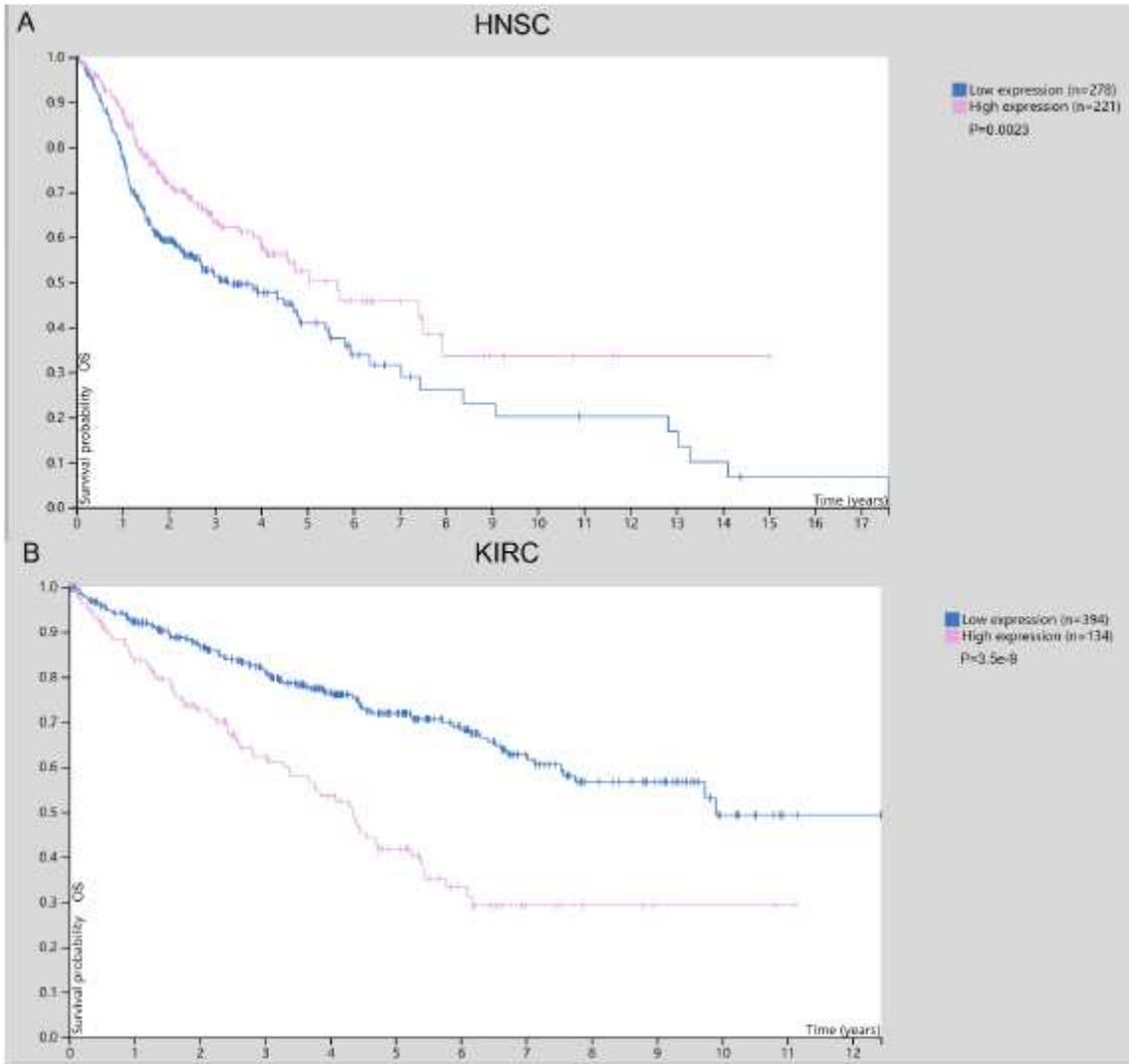


Figure 7

LPAR2 protein expression levels in HNSC and KIRC from the human protein atlas database (HPA). A: OS OF HNSC; B: OS OF KIRC.

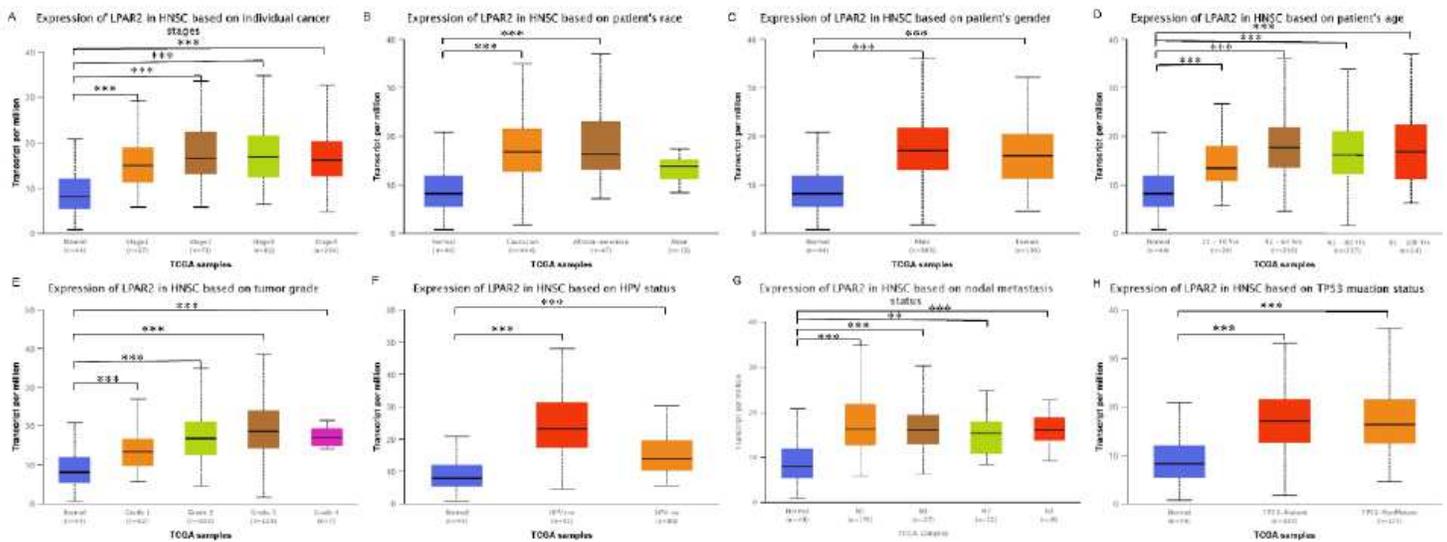


Figure 8

the relationship between the LPAR2 mRNA expression and clinical characteristics of HNSC patients from TCGA database in UCLAN. *P < 0.05, **P < 0.01, ***P < 0.001

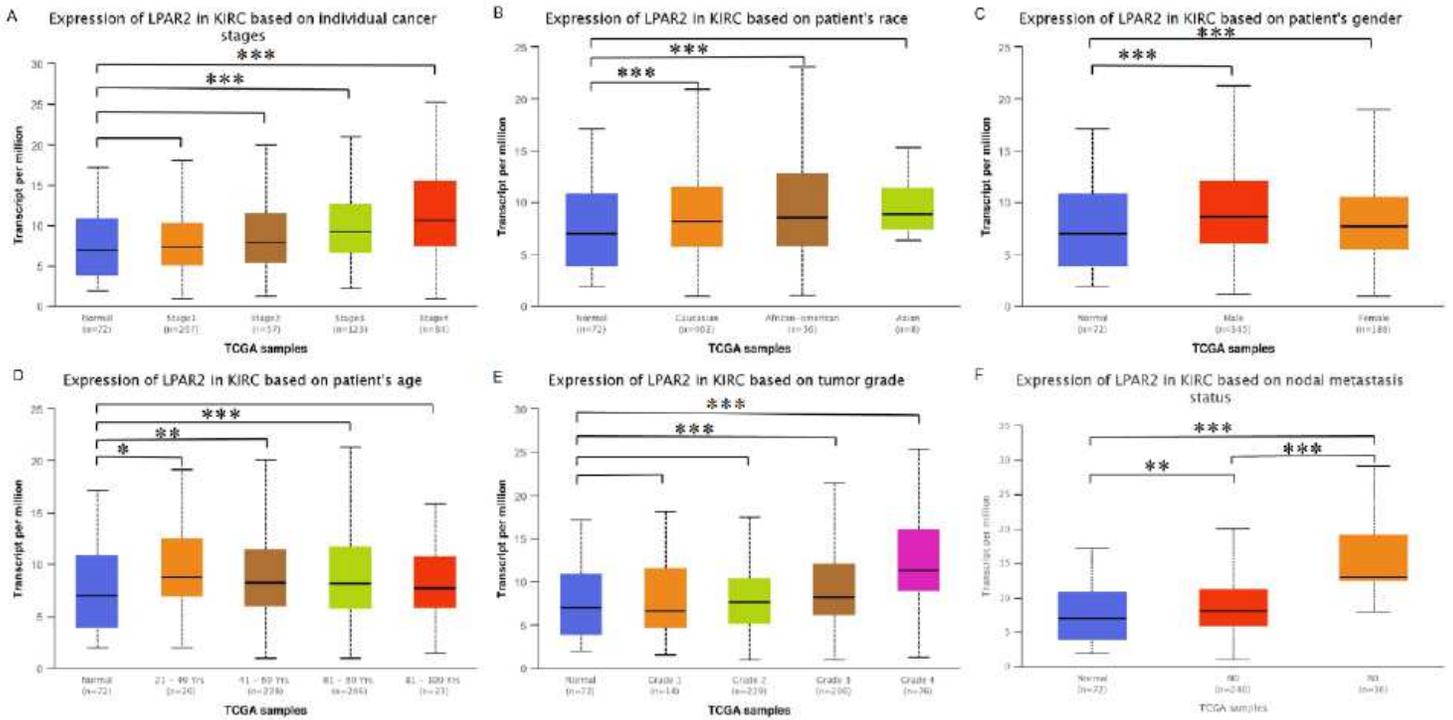


Figure 9

the relationship between the LPAR2 mRNA expression and clinical characteristics of KIRC patients from TCGA database in UCLAN. *P < 0.05, **P < 0.01, ***P < 0.001

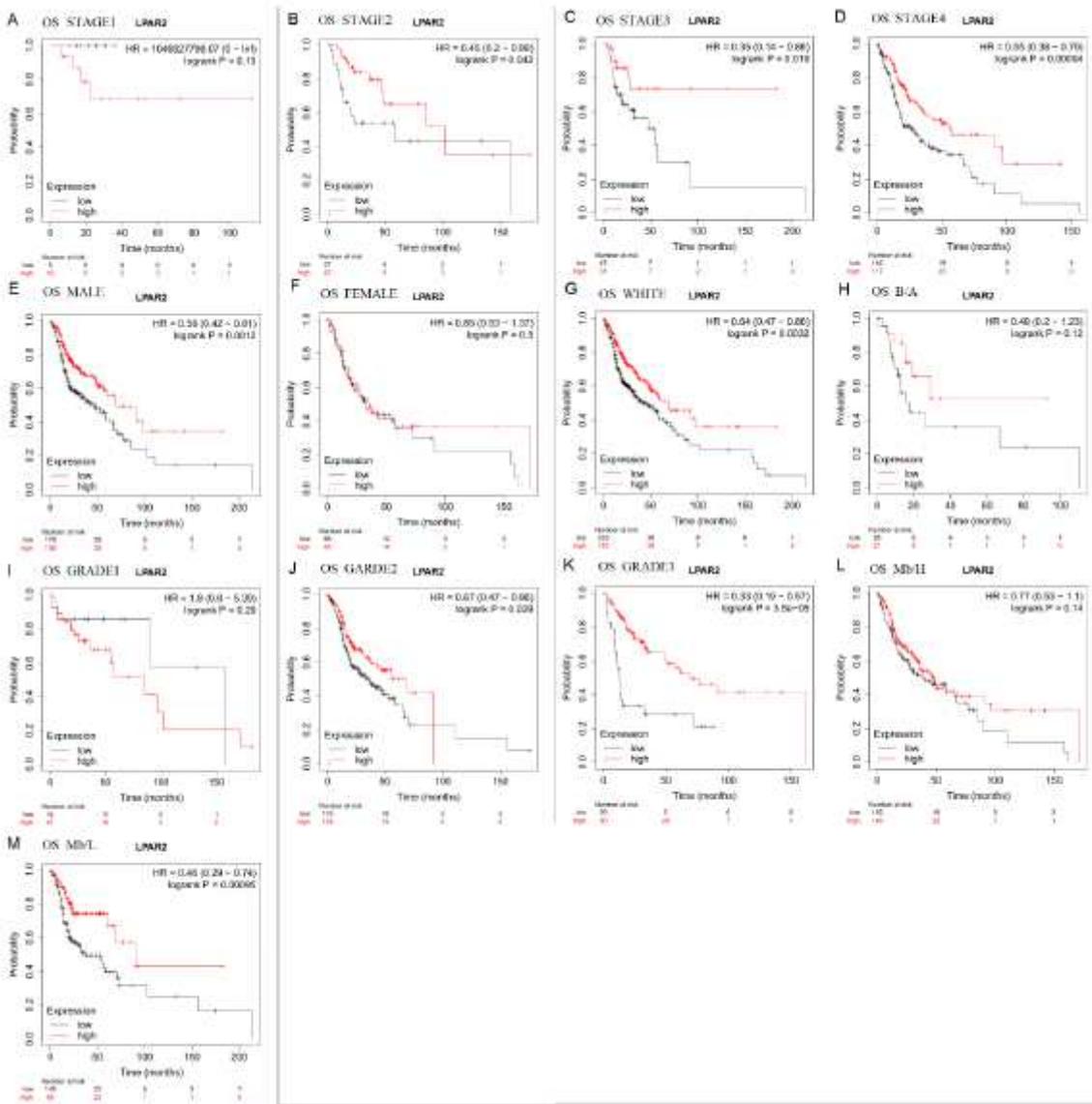


Figure 10

the relationship between the LPAR2 mRNA expression and prognosis in HNSC patients with different clinical characteristics in Kaplan-Meier plotter databases. 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

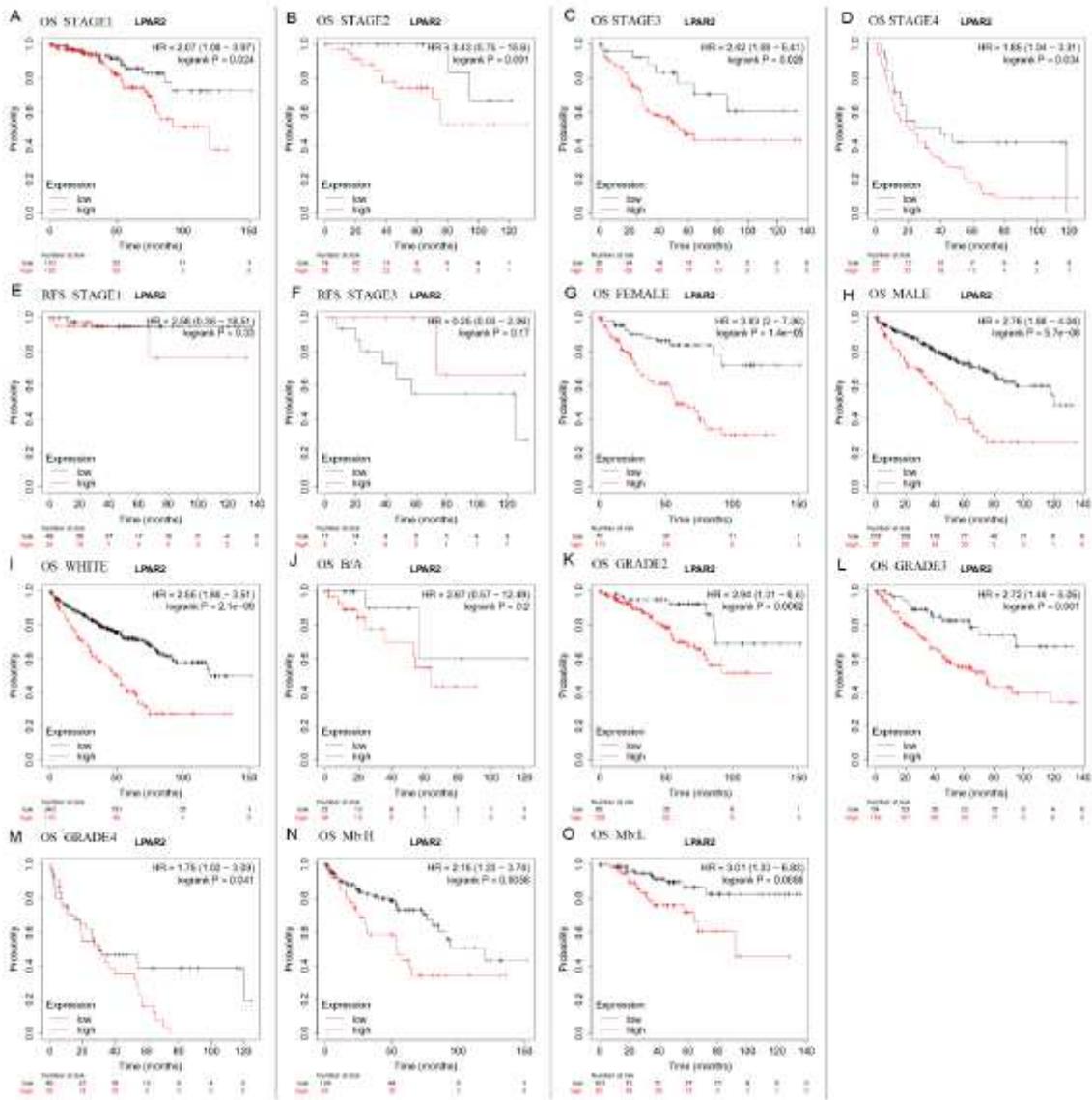


Figure 11

the relationship between the LPAR2 mRNA expression and prognosis in KIRC patients with different clinical characteristics in Kaplan-Meier plotter databases. 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

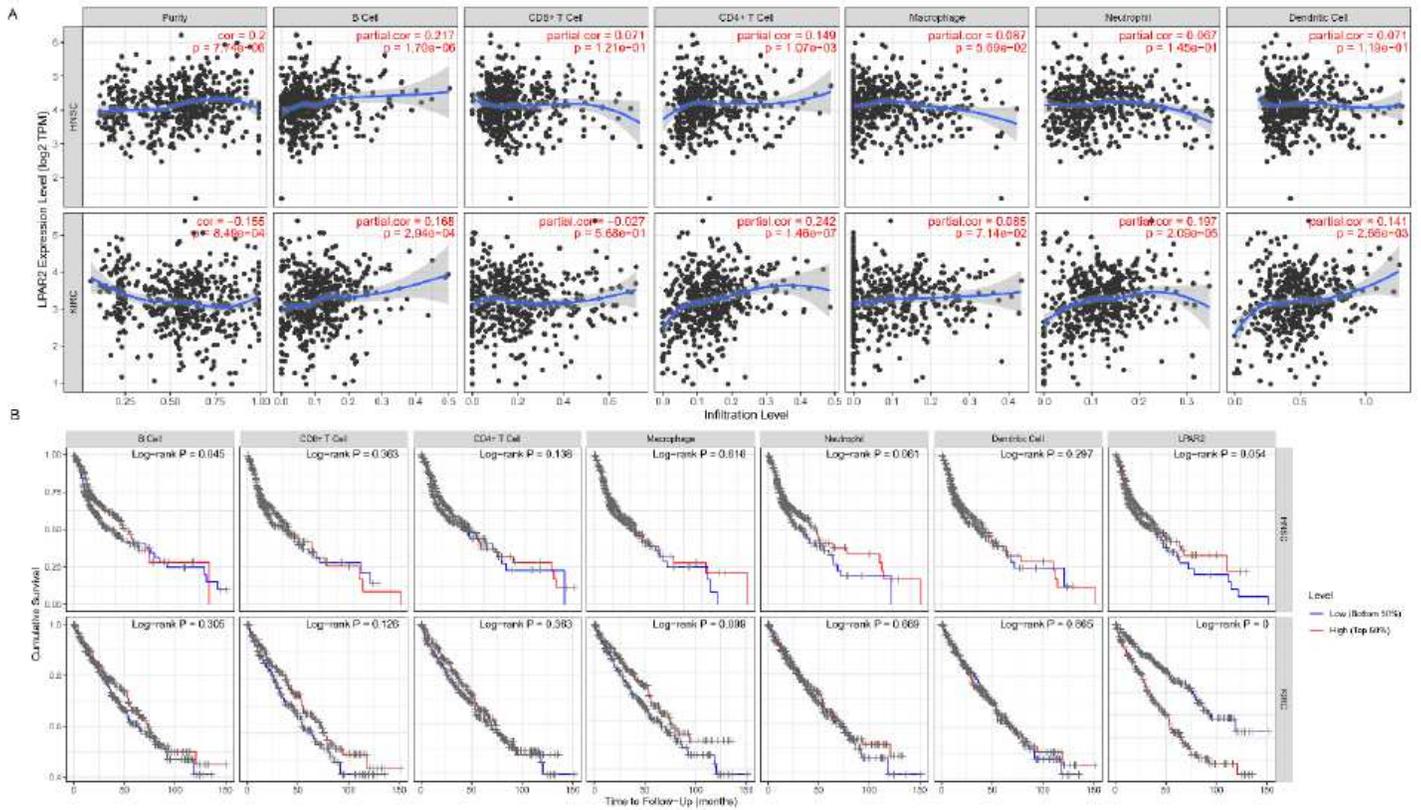


Figure 12

A) Correlation of LPAR2 expression with immune infiltration level in HNSC and KIRC. B) Kaplan-Meier plots of immune infiltration and LPAR2 expression levels in HNSC and KIRC.

