Research article

Prognostic and immunological value of LTB4R in pan-cancer

Sidan Long [1,2], Shuangshuang Ji [2], Kunmin Xiao [1,2], Peng Xue [1,*] and Shijie Zhu [1,*]

[1] Department of Oncology, Wangjing Hospital, China Academy of Chinese Medical Sciences, Beijing 100102, China.

[2] Graduate School, Beijing University of Chinese Medicine, Beijing, 100029, China.

* Correspondence: Email: xuepeng6399@163.com; 20180941234@bucm.edu.cn.

Abstract: *Background:* LTB4 receptor 1 (LTB4R), as the high affinity leukotriene B4 receptor, is rapidly revealing its function in malignancies. However, it is still uncertain. *Methods:* We investigated the expression pattern and prognostic significance of LTB4R in pan-cancer across different databases, including ONCOMINE, PrognoScan, GEPIA, and Kaplan-Meier Plotter, in this study. Meanwhile, we explored the significance of LTB4R in tumor metastasis by HCMDB. Then functional enrichment analysis of related genes was performed using GeneMANIA and DAVID. Lastly, utilizing the TIMER datasets, we looked into the links between LTB4R expression and immune infiltration in malignancies. *Results:* In general, tumor tissue displayed higher levels of LTB4R expression than normal tissue. Although LTB4R had a negative influence on pan-cancer, a high expression level of LTB4R was protective of LIHC (liver hepatocellular carcinoma) patients' survival. There was no significant difference in the distribution of LTB4R between non-metastatic and metastatic tumors. Based on Gene Set Enrichment Analysis, LTB4R was implicated in pathways involved in inflammation, immunity, metabolism, and cancer diseases. The correlation between immune cells and LTB4R was found to be distinct across cancer types. Furthermore, markers of infiltrating immune cells, such as Treg, T cell exhaustion and T helper cells, exhibited different LTB4R-related immune infiltration patterns. *Conclusion:* The LTB4R is associated with immune infiltrates and can be used as a prognostic biomarker in pan-cancer.

Keywords: leukotriene B4 receptor 1; pan-cancer; database; survival analysis; immune infiltration; tumor microenvironment

1. Introduction

Despite the use of a variety of cancer treatment options in the clinical context (e.g., surgery, chemotherapy, irradiation, and immunotherapy), cancer-related mortality remains one of the top causes of death worldwide, accounting for 13% of all human deaths [1]. Because cancer is considered a cell-intrinsic genetic illness, most treatment modalities are aimed at directly destroying tumor cells, with multidrug resistance of cancer cells being a major reason for cancer therapy's low efficacy [2].

Inflammation is now recognized as a characteristic of cancer, with multiple lines of evidence emphasizing the importance of chronic inflammation in fuelling tumor growth and modulating tumor response to treatment in various malignancies [1,3]. Leukotriene B4 (LTB4) is a key type of lipid mediator that is rapidly produced from arachidonic acid by the sequential activity of 5-lipoxygenase (5-LO). It has pleiotropic effects on numerous cells via interacting with the high affinity leukotriene B4 receptor 1 (LTB4R), the most well-known of which is the chemotactic action on neutrophils and macrophages [4]. However, its role in cancer is only beginning to emerge [5].

The abnormal expression of 5-LO was identified in numerous tumor cells, as was a rise in LTB4 levels in the tumor microenvironment (TME), indicating that LTB4 may play a role in cancer [6]. Besides neutrophils, recent studies have also discovered LTB4R expression on many cell types, including almost all immune cell types and some non-immune cells, such as macrophages, smooth muscle cells [7], endothelial cells [8], activated T-cells [9], mast cells [10], mature and immature dendritic cells [11], indicating a direct effect in the control of adaptive immune responses and significantly expanding LTB4's potential roles. This diversity of expression has led to the current concepts of yin and yang regulation of cancer as the activity of immune cells that influence both pro (neutrophils, MDSC) and anti-tumor (effector CD8+T cells) outcomes may be controlled by LTB4/BLT1 axis [12].

The TME is a complicated structure made up of tumor cells, blood vessels, extracellular matrix, and non-malignant cells [13]. Immune cells, which are non-malignant cells, play a crucial part in the TME. Increasing data suggest that interactions between tumor cells and the host immune system facilitate tumor immune escape, resulting in tumor spread, recurrence, and metastasis. In contrast, immunotherapy targeting interactions between immune cells and tumor cells has been developed in recent years as an alternate strategy to traditional anticancer treatments to reactivate adaptive and innate immune systems and build a robust antitumoral immune response. Tumor cells, for example, can upregulate programmed cell death 1 ligand expression, causing programmed cell death receptor 1-positive T cells to apoptosis, disablement, and depletion [14]. However, existing immunotherapies only work for a small percentage of individuals with specific cancer types. As a result, more possible targets must be investigated.

This study was conducted to comprehensively analyze LTB4R expression signature, prognostic value, correlation with tumor-infiltrating immune cells, and associated pathways using data from public platforms. Our research aims to provide more information to better understand the significance of LTB4R in various cancers.

2. Materials and methods

2.1. LTB4R expression in human cancers in ONCOMINE

The ONCOMINE database was used to examine the mRNA expression of LTB4R in various

cancer types (www.oncomine.org) [15]. The thresholds were set as a P-value of 0.001 and fold change of 1.5.

2.2. Metastatic potential of LTB4R in HCMDB

The Human Cancer Metastasis Database (HCMDB https://hcmdb.i-sanger.com/) is a freely available online tool for assessing gene metastatic potential. The interactive web tool contains metastatic data from 38 metastasis sites and 29 cancer types [16]. In the present study, the HCMDB database was used to test the metastatic potential of LTB4R in TCGA-CESC data. We used the meta-analysis function of stataSE 16 to analyze all statistically different data sets individually, with using the random effects model for continuous variables of LTB4R expression.

2.3. Survival analysis in PrognoScan, GEPIA, and Kaplan-Meier Plotter

The correlation between LTB4R expression and survival in pan-cancer was analyzed in PrognoScan [17] (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html), Kaplan-Meier Plotter [18] (https://kmplot.com/analysis/), and GEPIA [19] (http://gepia.cancer-pku.cn/). The LTB4R expression level was specifically examined in all accessible PrognoScan microarray datasets to establish its association with prognosis, including overall survival (OS) and disease-free survival (DFS). A Cox P-value of 0.05 was used as the cutoff. GEPIA is an interactive online platform that contains tumor sample data from the TCGA as well as normal sample data from the TCGA and GTEx studies. We investigated the impact of LTB4R expression on OS and DFS in each cancer type (total number = 33). The Kaplan-Meier Plotter is a powerful online tool that may be used to evaluate the impact of 54,000 genes on survival in 21 different cancer types. We investigated the connection between LTB4R expression and OS and relapse-free survival (RFS). Log-rank P-values and hazard ratios (HRs) with 95 percent confidence intervals (CI) were computed.

2.4. GeneMANIA analysis and functional enrichment analysis

LTB4R co-expression genes were analyzed using the GeneMANIA (http://www.genemania.org) [20], a user-friendly website, can display gene or gene lists that share the same functions as submitted genes and provide a PPI network. The genes interacting with LTB4R, as determined by GeneMANIA, were all input into Metascape [21] for further functional annotations and analyses.

2.5. GO and KEGG pathway analyses

DAVID [22] (https://david.ncifcrf.gov/) is an integrated online database that can provide researchers with comprehensive gene functional annotation information to help them understand biological processes. GO analysis annotates genes and analyzes the molecular function (MF), biological process (BP) and cellular component (CC) of these genes. KEGG is another powerful database for the functional interpretation of genomic sequences. We used the DAVID database to analyze the GO and KEGG pathways of the related genes. All significant GO and KEGG enrichment pathways were visualized with the ggplot package.

2.6. Correlation analysis between LTB4R and immune infiltrates by tumor immunity estimate resource

The relationship between LTB4R expression and immune infiltration was determined using the TIMER [23] (http://cistrome.org/TIMER/). The TIMER database comprises 10,897 TCGA samples from 32 cancer types to assess the quantity of immune infiltration, making it an appropriate resource for systematic investigation of immune infiltration across varied cancer types. We investigated the relationship between LTB4R expression and the abundance of all six categories of immune invading cells, including B cells, CD4+T cells, CD8+T cells, neutrophils, macrophages, and dendritic cells. The relationship between the expression level of LTB4R and tumor purity was also determined to can reduce analysis bias [24].

In addition to the broad examination of immune cell type, we looked at the relationship between LTB4R expression and a number of immune cell markers to identify potential subgroups of invading immune cells. Immune gene markers were chosen from Cell Signaling Technology (https://www.cst-c.com.cn/common/content/content.jsp?id=pathways-ti-icm-human) and R&D Systems. (https://www.rndsystems.com/cn/resources/cell-markers/immune-cells). These gene markers include markers of B cells, CD8+T cells, follicular helper T cells (Tfh), T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, T-helper 9 (Th9) cells, T-helper 17 (Th17) cells, T-helper 22 (Th22) cells, Tregs, exhausted T cells, M1 macrophages, M2 macrophages, tumor-associated macrophages (TAM), monocytes, natural killer (NK) cells, neutrophils, dendritic cells (DC) and regulatory B cells (Breg).

2.7. Statistical analysis

LTB4R expression levels were determined using Oncomine and GEPIA. Oncomine results are given with P-values, fold changes, and ranks. P-values are represented by asterisks in the GEPIA results. Survival curves were obtained using PrognoScan, Kaplan–Meier Plotter, and GEPIA; the findings are given with HR and P-values or Cox P-values from log-rank testing. Spearman's correlation was used to assess the correlation of gene expression. A P-value of less than 0.05 was considered to indicate a significant difference.

3. Results

3.1. Pan-cancer messenger RNA expression levels of LTB4R

First, we examined the messenger RNA expression levels of LTB4R in various malignancies using the Oncomine database. The findings demonstrated that, as compared to the corresponding normal groups, LTB4R expression was higher in cancer groups, including colorectal, head and neck, and lymphoma. Meanwhile, in one head and neck cancer dataset, LTB4R expression was found to be lower (Figure 1A).

We used TIMER to investigate RNA sequencing data from the TCGA to further evaluate LTB4R expression in pan-cancer. Figure 1B depicts the differential LTB4R expression patterns in tumor and neighboring normal tissues. LTB4R expression was significantly lower in PRAD (prostate adenocarcinoma) and THCA (thyroid carcinoma) than in normal tissue. Meanwhile, LTB4R expression was significantly higher in BLCA (bladder urothelial carcinoma), CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), CHOL (cholangiocarcinoma), COAD (colon adenocarcinoma), ESCA (esophageal carcinoma), GBM (glioblastoma multiforme), KIRC

(kidney renal clear cell carcinoma), KIRP (kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), LUSC (lung squamous cell carcinoma), READ (rectum adenocarcinoma), STAD (stomach adenocarcinoma) than in their respective adjacent normal tissues.
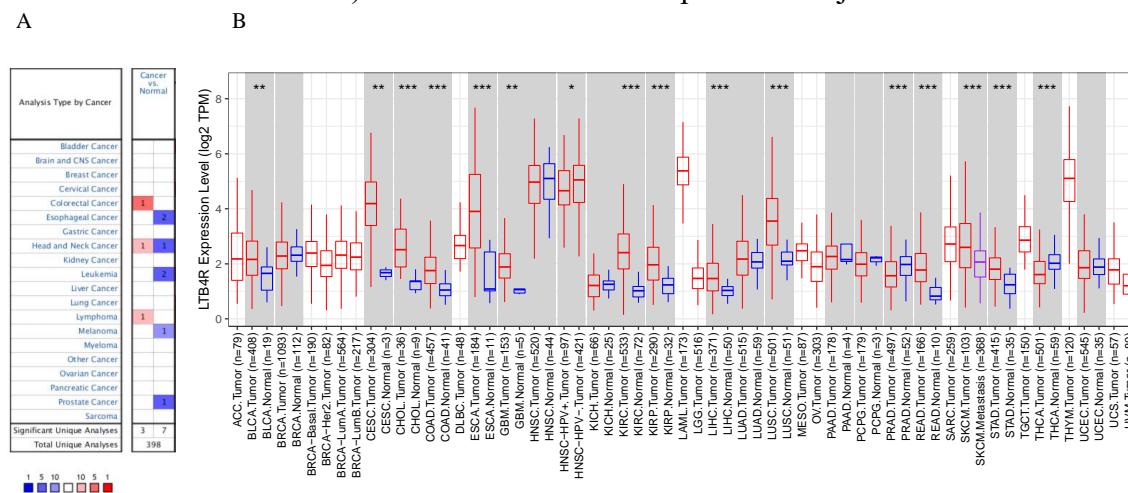

Figure 1. LTB4R expression levels in cancers. (A) Increased or decreased expression of LTB4R in different cancer tissues, compared with normal tissues in Oncomine. Number in each cell is the amounts of datasets. (B) Human LTB4R expression levels in different cancer types from TCGA data in TIMER (< 0.1, *P < 0.05, **P < 0.01, ***P < 0.001).
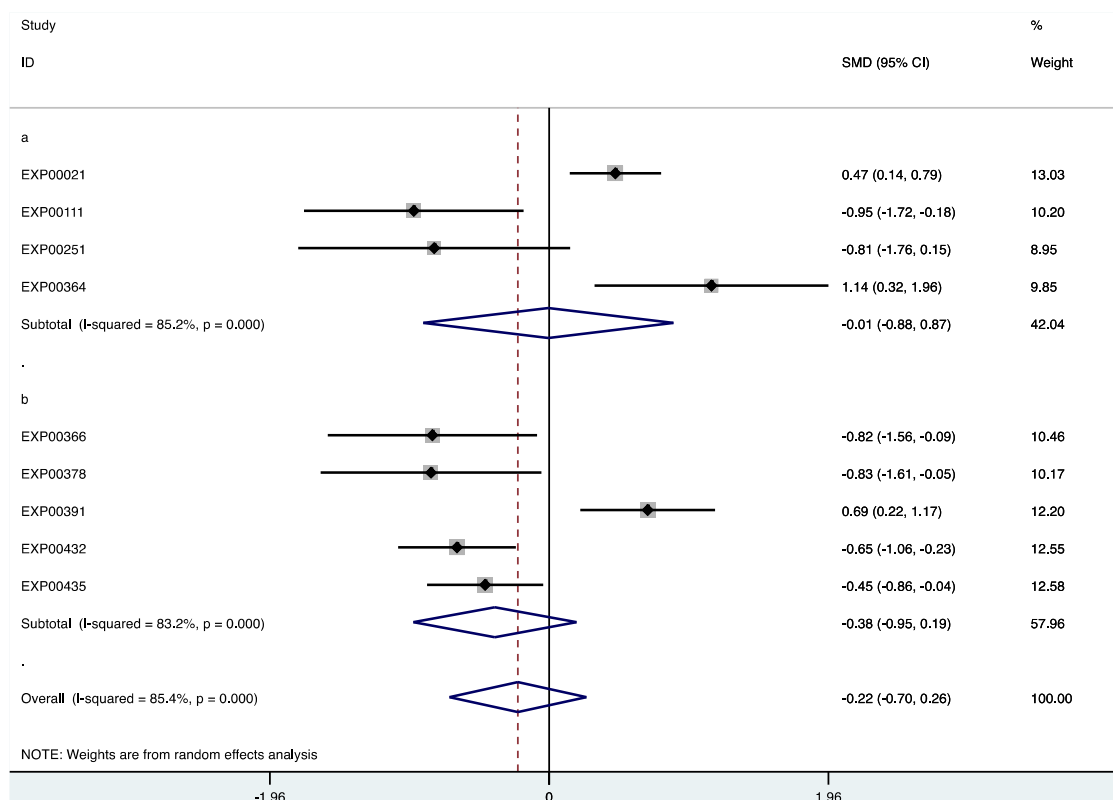

Figure 2. Exploring the differences in LTB4R expression levels at primary tumor sites in patients with different metastatic burden using the HCMDB database and meta-analysis. (a) Primary cancer: colorectal cancer (b) Other primary tumors.

Table 1. Comparison of LTB4R expression levels in primary tumors from patients with metastatic and non-metastatic in HCMDB.

Exp ID	Cancer type	Primary site	Metastasis site	Design	Sample	Log2FC	P-value
EXP00021	colorectal cancer	colorectum	liver	[primary tumors with metastasis vs. primary tumor without metastasis] of colorectal cancer	189	0.521	6.022e-3
EXP00111	colorectal cancer	colorectum	liver	[primary tumors with metastasis vs. primary tumor without metastasis] of colorectal cancer	30	-0.273	2.119e-2
EXP00251	colorectal cancer	colorectum	liver	[primary tumors with metastasis vs. primary tumor without metastasis] of colorectal cancer	20	-0.213	2.563e-2
EXP00364	colorectal cancer	colorectum	liver,ovary,peritoneum	[primary tumors with metastasis vs. primary tumor without metastasis] of colorectal cancer	27	0.587	8.374e-3
EXP00366	skin cancer	skin	skin	[primary tumors with metastasis vs. primary tumor without metastasis] of skin cancer	42	-0.986	1.55e-3
EXP00378	eye cancer	eye	unknown	[primary tumors with metastasis vs. primary tumor without metastasis] of eye cancer (uveal melanoma)	28	-0.125	3.701e-2
EXP00391	kindey cancer	adrenal gland	bone, liver, lung, lymph node, other, peritoneal surfaces, soft tissue	[primary tumors with metastasis vs. primary tumor without metastasis] of kindey cancer (adrenocortical carcinoma)	79	0.637	3.701e-3
EXP00432	Esophageal cancer	esophagus	bone, brain, liver, lung, etc	[primary tumors with metastasis vs. primary tumor without metastasis] of esophagus cancer (esophageal carcinoma)	161	-0.86	1.358e-3
EXP00435	pancreatic cancer	pancreas	liver, lung, peritoneal surfaces	[primary tumors with metastasis vs. primary tumor without metastasis] of pancreatic cancer (pancreatic adenocarcinoma)	169	-0.272	3.475e-2

3.2. Evaluation of metastatic potential of LTB4R

We explored the role of LTB4R in pan-cancer metastasis using the HCMDB database, and results with statistical differences are visible in Table 1. By comparing the expression levels of LTB4R in the primary tumor sites of patients with and without metastasis in pan-cancer (SMD = -0.22; 95%CI −0.70, 0.26; P = 0.369〉, and after further subgroup analysis according to the type of primary tumor (SMD = -0.01; 95%CI −0.88, 0.87; P = 0.99〉, we found that LTB4R was not a good predictor of distant tumor metastasis (Figure 2).
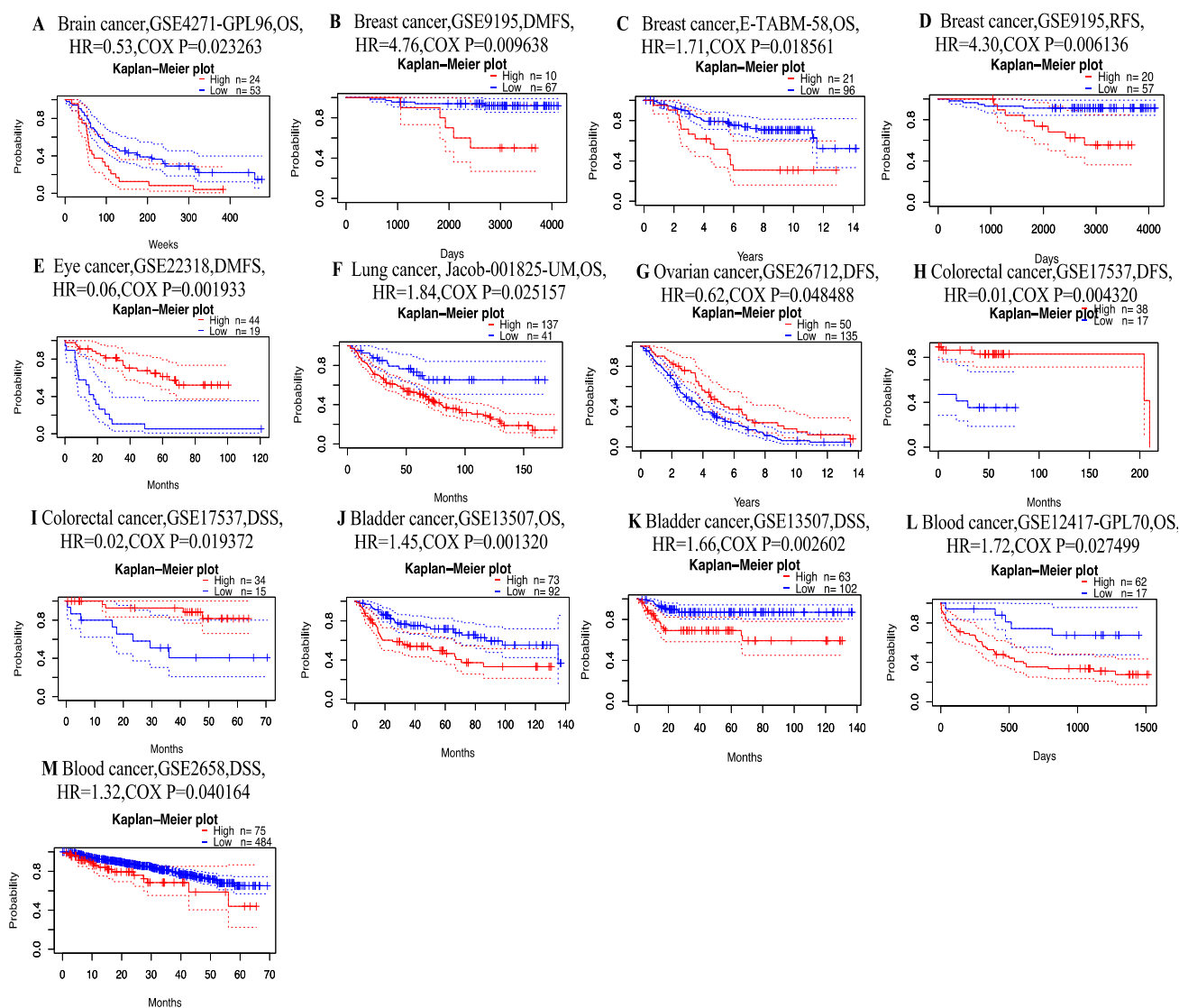

Figure 3. Survival curves comparing high and low expression of LTB4R in different cancer types in PrognoScan. OS, overall survival; DMFS, distant metastasis-free survival; RFS, relapse-free survival; DSS, disease-specific survival. (A) OS of Brain Cancer. (B–D) DMFS, OS, and RFS of Breast Cancer. (E) DMFS of Eye Cancer. (F) OS of Lung Cancer. (G) DFS of Ovarian Cancer. (H–I) DFS, DSS of colorectal cancer. (J–K) OS, DSS of bladder cancer. (L–M) AML-OS, MM-DSS of blood cancer.

3.3 Multifaceted prognostic value of LTB4R in cancers

Then, utilizing several datasets, we examined LTB4's pan-cancer prognostic value. We investigated the association between BLT1 expression and cancer prognosis in PrognoScan; the results are accessible here: http://dna00.bio.kyutech.ac.jp/PrognoScan-cgi/PrognoScan.cgi Notably, LTB4R expression was found to be substantially associated to the prognosis of eight different cancer types (Figure 3): breast, eye, lung, brain, ovarian, bladder, colorectal cancers and blood cancer: AML (acute myeloid leukemia), MM (multiple myeloma).

Among them, LTB4R had a protective effect in three types of cancer: eye [DMFS: total number = 63, HR = 0.06, Cox P = 0.001933], colorectal [DFS: total number = 55, HR = 0.01, Cox P = 0.004320; DSS: total number = 49, HR = 0.02, Cox P = 0.019372], ovarian [DFS: total number = 185, HR = 0.62, Cox P = 0.048488]. Meanwhile, LTB4R had a detrimental role in the other 5 cancer types, including breast [DMFS: total number = 77,HR = 4.76,Cox P = 0.009638; RFS: total number = 77, HR = 4.30, Cox P = 0.006136; DSS: total number = 117, HR = 1.80, Cox P = 0.018549; OS: total number = 117, HR = 1.71, Cox P = 0.018561], lung adenocarcinoma (LUAD) [OS: total number = 178, HR = 1.84, Cox P = 0.025157], brain [OS: total number = 77, HR = 1.53, Cox P = 0.023263], bladder [OS: total number = 165, HR = 1.45, Cox P = 0.001320; DSS: total number = 165, HR = 1.66, Cox P = 0.002602], blood cancer [AML-OS: total number = 79, HR = 1.72, Cox P = 0.027499; MM-DSS: total number = 559, HR = 1.32, Cox P = 0.040164].

Following that, we investigated the prognosis survival of LTB4R-related malignancies using the Kaplan–Meier Plotter, which mostly employs TCGA data (in contrast to PrognoScan, whose data mainly comes from the Gene Expression Omnibus database). We showed for the first time that LTB4R is related to poor prognosis in KIRP (OS: HR = 2.7, log-rank P = 0.00074; RFS: HR = 2.1, log-rank P = 0.048) (Figures 4 A, B). At the same time, we newly identified LTB4R as a protective prognostic factor in PDAC (pancreatic ductal adenocarcinoma) (OS: HR = 0.43, log-rank P = 0.00019; RFS: HR = 0.31, log-rank P = 0.003) (Figures 4 C, D). The results for BC (bladder cancer) and BRCA (breast cancer) were partially different from those obtained in PrognoScan: LTB4R had a positive impact on OS (HR = 0.67 , log-rank P = 0.041) but not on RFS (HR = 1.68 , log-rank P = 0.054) in BRCA (Figures 4 E,F);LTB4R did not have a significant effect on overall survival and relapse-free survival in BC (OS: HR = 0.75, log-rank P = 0.082; RFS: HR = 0.65,log-rank P = 0.23) (Figures 4 G,H); For both liver hepatocellular carcinoma (LIHC) and LUAD, LTB4R significantly influenced their overall survival (LIHC, OS, HR = 0.65, log-rank P = 0.02; LUAD, OS, HR = 0.64 , log-rank P = 0.014), but not relapse-free survival (LIHC, RFS, HR = 1.22, log-rank P = 0.25; LUAD, RFS, HR = 1.35, log-rank P = 0.19) (Figures 4 I-L). Interestingly, unlike LUAD, LTB4R did not have a significant effect on overall survival and relapse-free survival in LUSC (OS, HR = 0.81, log-rank P = 0.18; RFS, HR = 0.61, log-rank P = 0.064). For Ovarian cancer (OC), LTB4R had a protective effect on relapse-free survival (RFS: HR = 0.45, log-rank P = 2.7e-05) but did not have a significant effect on overall survival (OS: HR = 0.85, log-rank P = 0.22) (Figures 4 M, N). In addition, for colorectal cancer, LTB4R did not have a significant effect on overall survival and relapse-free survival in rectum adenocarcinoma (READ) (OS: HR = 1.71, log-rank P = 0.22; RFS: HR = 3.68, log-rank P = 0.11) (Figures 4 O, P).

In addition to the LTB4R microarray analysis in PrognoScan and Kaplan-Meier Plotter, we used GEPIA to analyze TCGA RNA sequencing data. In GEPIA, we looked at the involvement of LTB4R in each cancer type (33 total), as well as the overall influence of LTB4R on malignancies. In general, LTB4R was a deleterious prognostic marker in cancers (OS: total number = 9,488, HR = 1.2, log-rank

P = 6.4e-06; RFS: total number = 9,488, HR = 1, log-rank P = 0.79) Specifically, compared with a low expression level, a high expression level of LTB4R was correlated with a better OS in PAAD and uveal melanoma (UVM) and RFS in PAAD. On the contrary, compared with a low expression level, a high expression level of LTB4R was correlated with a poorer OS in ACC, KIRC, KIRP, LAML, LGG. Unlike the findings from PrognoScan and Kaplan-Meier Plotter, LTB4R expression impacted not OS in BRCA and LUAD. In addition, we did not find any effects of LTB4R on LUSC, READ, etc. (Figure 5） These results confirmed the prognostic value of LTB4R in some specific types of cancers and that increased and decreased LTB4R expression have different prognostic value depending on the type of cancer.
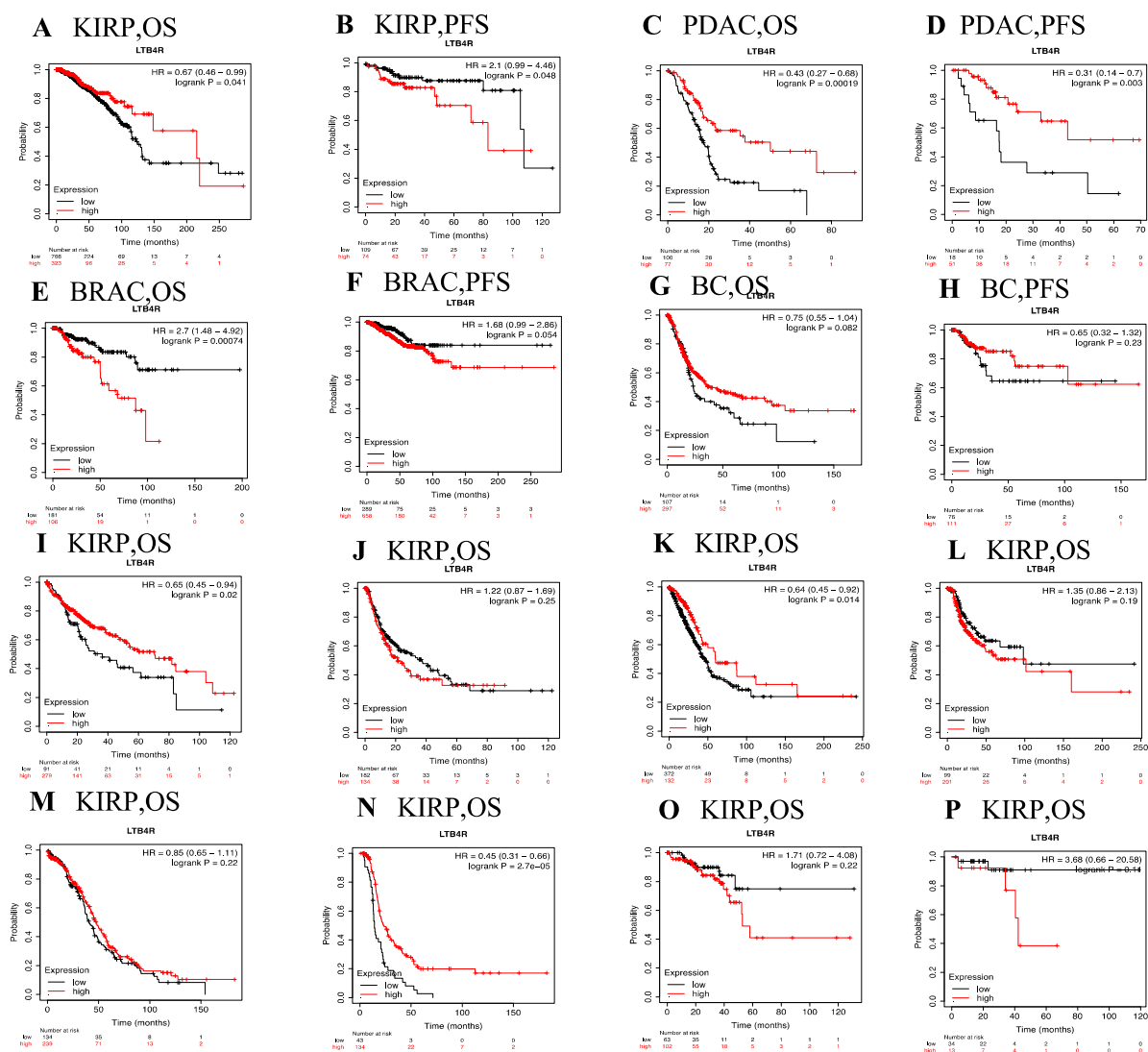

Figure 4. Kaplan-Meier survival curves comparing the high and low expression of LTB4R in different types of cancer in Kaplan-Meier Plotter. OS and RFS of (A, B) kidney renal papillary cell carcinoma (KIRP); (C, D) pancreatic ductal adenocarcinoma (PDAC); (E,F) breast cancer (BRCA); (G,H) bladder cancer (BC); (I,J) liver hepatocellular carcinoma(LIHC); (K,L) lung adenocarcinoma(LUAD); (M,N) Ovarian cancer (OC); and (O,P) rectum adenocarcinoma (READ). The red curve represents patients with high expression of LTB4R. OS, overall survival; RFS, relapse-free survival.

Table 2. Correlation of LTB4R messenger RNA expression with OS (n = 364) and PFS (n = 366) in LIHC with different clinicopathological features.

Clinicopathological features	OS (n = 364)			PFS (n = 366)		
	N	Hazard ratio	P	N	Hazard ratio	P
Stage						
1	170	0.55（0.24-1.24）	0.14	170	1.47（0.89-2.43）	0.13
1+2	253	0.58（0.35-0.95）	0.029*	254	1.39（0.95-2.04）	0.088
2	83	0.49 (0.23-1.07)	0.067	84	1.89（1.02-3.49）	0.039*
2+3	166	0.58（0.36-0.93）	0.021*	167	1.33（0.88-1.99）	0.17
3	83	0.51（0.25-1.06）	0.067	83	0.68（0.36-1.28）	0.23
3+4	87	0.49（0.24-1.02）	0.05	88	0.55（0.31-0.96）	0.032*
4	4	—	—	5	—	—
Grade						
1	55	1.47 (0.57-3.8)	0.43	55	0.34 (0.15-0.77)	0.0068*
2	174	0.54 (0.29-1.02)	0.052	175	1.51（0.97-2.34）	0.063
3	118	0.46 (0.24-0.88)	0.017*	119	0.55（0.31-0.96）	0.033*
4	12	—	—	12	—	—
AJCC_T						
1	180	0.73（0.38-1.38）	0.33	180	1.48（0.91-2.41）	0.11
2	90	0.49（0.24-1.02）	0.051	92	1.49（0.85-2.63）	0.16
3	78	0.54（0.26-1.14）	0.1	78	1.51（0.75-3.03）	0.24
4	13	—	—	13	—	—
Vascular invasion						
None	203	0.64（0.37-1.1）	0.1	204	0.8（0.5-1.28）	0.35
Micro	90	0.46（0.21-0.99）	0.043*	91	1.74（0.97-3.14）	0.062
Macro	16	—	—	16	—	—
Sex						
Male	246	0.54（0.34-0.84）	0.0055*	246	0.75（0.51-1.11）	0.15
Female	118	0.58（0.29-1.17）	0.12	120	1.36（0.81-2.27）	0.24
Race						
White	181	0.66（0.41-1.05）	0.078	183	0.65（0.43-0.97）	0.034*
Black of African American	17	—	—	17	—	—
Asian	155	0.5（0.27-0.93）	0.027*	155	1.53（0.95-2.46）	0.076
Sorafenib treatment						
Treated	29	0.44（0.13-1.48）	0.17	30	0.39（0.17-0.92）	0.027*
Alcohol consumption						
Yes	115	0.45（0.23-0.85）	0.012*	115	0.56（0.32-0.98）	0.039*
None	202	0.63（0.39-1.02）	0.056	204	1.52（1-2.3）	0.048*
Hepatitis virus						
Yes	150	0.6（0.31-1.17）	0.13	152	1.63（1.02-2.26）	0.04*
None	167	0.53（0.33-0.84）	0.0068*	167	0.67（0.41-1.08）	0.098

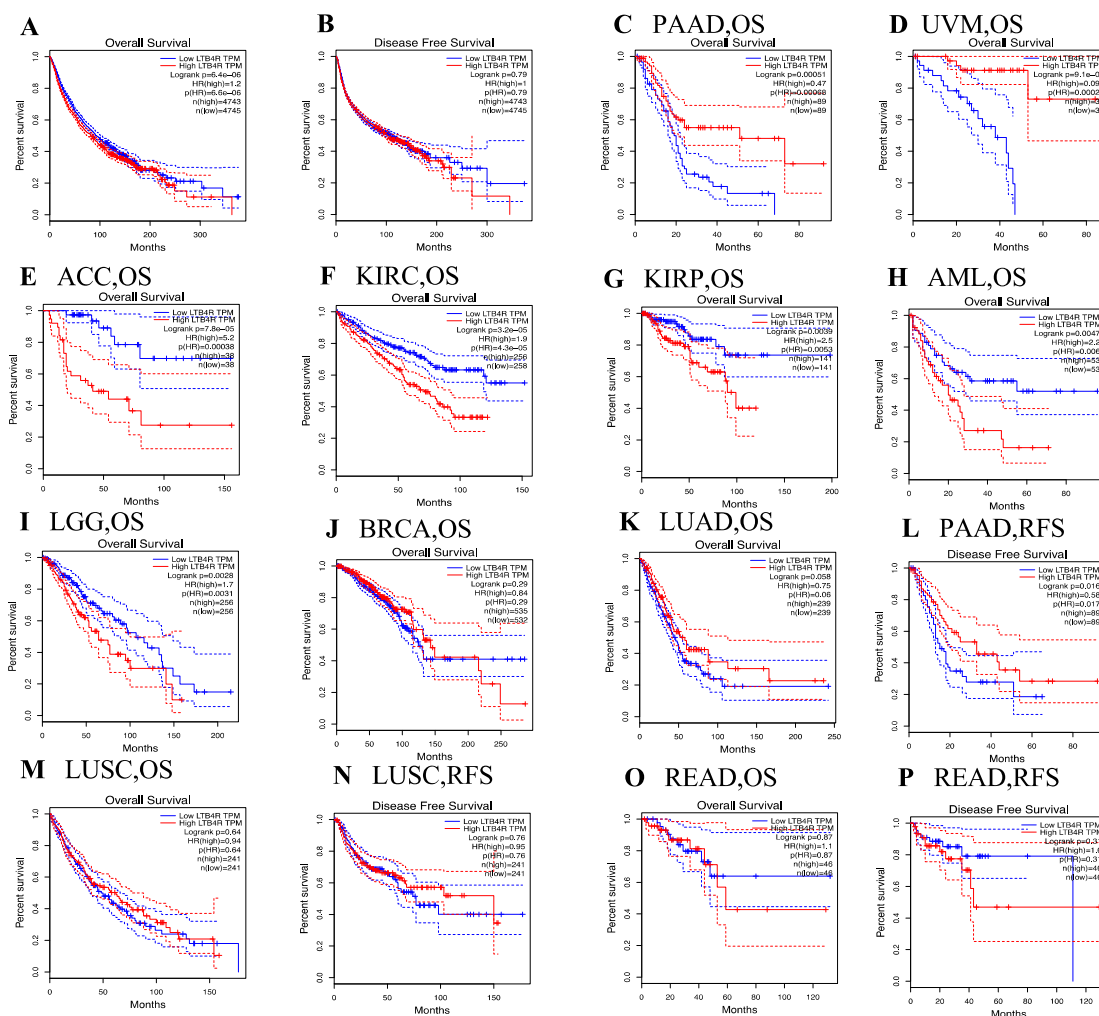OS, overall survival; PFS, progression-free survival. *$P < 0.05$.

Figure 5. Survival curves comparing the high and low expression of LTB4R in different types of cancer in GEPIA. The red curve represents patients with high expression of LTB4R. OS, overall survival; RFS, relapse-free survival.

3.4. LTB4R expression in a stratified population

We used the Kaplan–Meier Plotter database to study the association between LTB4R expression and clinical features of LIHC patients in order to better understand the influence of LTB4R on cancer patient survival (Table 2). For OS, LTB4R played a protective role in patients with LIHC with the following characteristics: stage1+2 (HR = 0.58, P = 0.029), stage2+3 (HR = 0.58, P = 0.021), grade 3 (HR = 0.46, P = 0.017 ）, microvascular invasion (HR = 0.46, P = 0.043), male (HR = 0.54 , P = 0.0055), Asian (HR = 0.5, P = 0.027), alcohol consumption (HR = 0.45, P = 0.012), no hepatitis virus infection (HR = 0.53, P = 0.0068). For progression-free survival (PFS), LTB4R expression was significantly hazardous to LIHC patients with no-alcohol consumption (HR = 1.52, P = 0.048), hepatitis virus infection (HR = 1.63, P = 0.04), or in stage 2 (HR = 1.89, P = 0.039). On the contrary, LTB4R had benefit in stage 3+4 (HR = 0.55, P = 0.032), grade 1 (HR = 0.34, P = 0.0068) or grade 3 (HR = 0.55, P = 0.033) patients, those with alcohol consumption (HR =0.56, P = 0.039), white patients (HR = 0.65, P = 0.034), or those undergoing sorafenib treatment (HR = 0.39, P = 0.027).

3.5. Functions and mechanisms of LTB4R and its related genes

Figure 6A shows the interaction network and biological functions of LTB4R and its related genes. LTB4R was found to be involved in multiple biological processes, such as GPCR downstream signaling, PID CXCR4 pathway, cardiac muscle contraction, glioblastoma signaling pathway, maintenance of location in cell, regulation of cysteine-type endopeptidase activity, maintenance of location in cell and cellular response to abiotic stimulus (Figure 6B）.
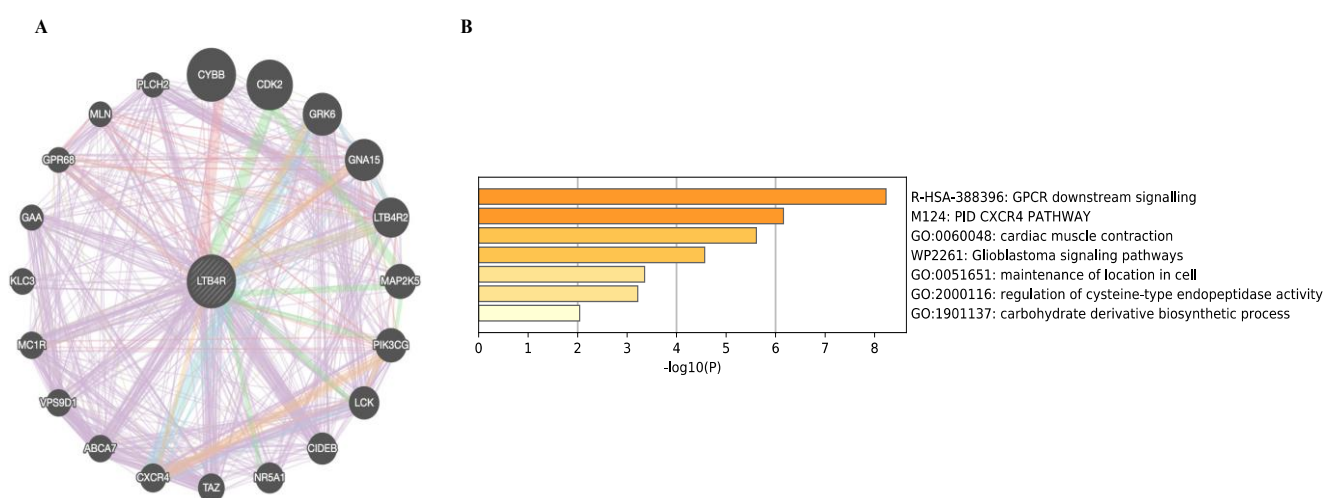

Figure 6. (A) The interaction network of LTB4R and its related genes; (B) The biological functions of LTB4R and its related genes analyzed by Metascape.

3.6. GO and KEGG enrichment pathways of the related genes

The GO analysis results showed that the genes were significantly enriched in the biological processes of inflammatory response, platelet activation, positive regulation of cytosolic calcium ion concentration, G-protein coupled receptor signaling pathway, innate immune response, protein phosphorylation etc. (Figure 7A). Changes in cellular components were significantly enriched in plasma membrane (Figure 7B). With regard to molecular function, genes were mainly enriched in ATP binding, kinase activity, protein kinase activity, protein serine/threonine kinase activity, etc (Figure 7C). KEGG pathway analysis showed that LTB4R and its related genes were mainly enriched in osteoclast differentiation and chemokine signaling pathway (Figure 7D).

3.7. The levels of LTB4R expression correlate with the infiltration levels of immune cells

Aside from prognostic value, the connection between LTB4R changes and six immunological infiltrative cells (B cells, CD4^{+}T cells, CD8^{+}T cells, dendritic cells, macrophages, and neutrophils) across different cancer types was investigated. LTB4R expression was found to be substantially linked with the six immune infiltrates in the majority of cancer types (supplementary files).
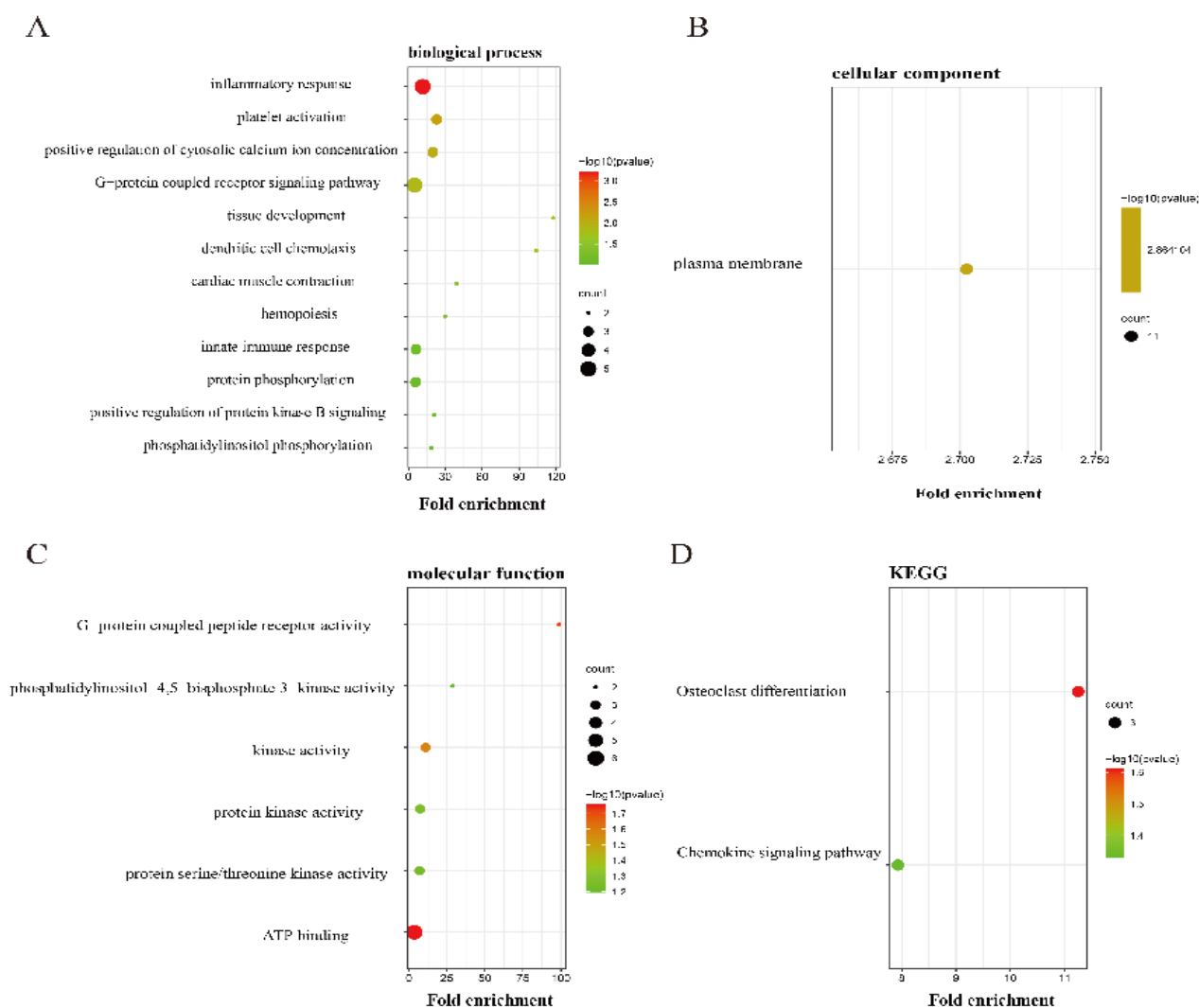
Figure 7. Significant GO and KEGG enrichment pathways of LTB4R. (A) BP terms of GO. (B) CC terms of GO. (C) MF terms of GO. (D) KEGG pathways.

Based on the above findings in GEPIA, PrognoScan and Kaplan-Meier Plotter, we chose KIRP and LGG to represent cancers with poor survival, PAAD and LIHC to represent cancers with good survival when LTB4R had a high expression level.

For PAAD, the LTB4R expression level had significant positive correlations with the infiltration levels of CD4+T cells (R = 0.441, P = 2.03e-09), neutrophils (R = 0.19, P = 1.3e-02). For LIHC, the LTB4R expression level had significant positive correlations with the infiltration levels of B cell (R=0.129, P = 1.65e-02), CD8+T cells (R = 0.129, P =1.67e-02), CD4+T cells (R =0.302, P =1.04e-08), macrophages cell (R = 0.213, P = 7.14e-5), neutrophils cell (R = 0.229, P = 1.77e-05), dendritic cells (R = 0.215, P = 6.49e-5) with tumor purity (R = 0.178, P = 8.65e-04).

For LGG, the LTB4R expression level had significant negative correlations with the infiltration levels of CD8$^+$T cells (R = -0.171, P = 1.65e-04), while had significant postive correlations with the infiltration levels of CD4+T cells (R = 0.264, P = 5.25e-09), macrophages cell (R = 0.22, P = 1.3e-06), neutrophils cell (R = 0.137, P = 2.82e-03), dendritic cells (R = 0.13, P = 4.45e-03) with tumor purity (R = 0.092, P = 4.40e-2). For KIRP, the LTB4R expression level had significant positive correlations with the infiltration levels of CD4+T cells (R = 0.36, P = 2.65e-09) and neutrophils cell (R = 0.309, P

= 4.3e-07) (Figure 8). Although these findings show variation between the status of tumor infiltration of immune cells, the level of LTB4R expression and prognosis in different cancers, the data suggest that LTB4R expression modulates infiltration of immune cells into tumor tissues.
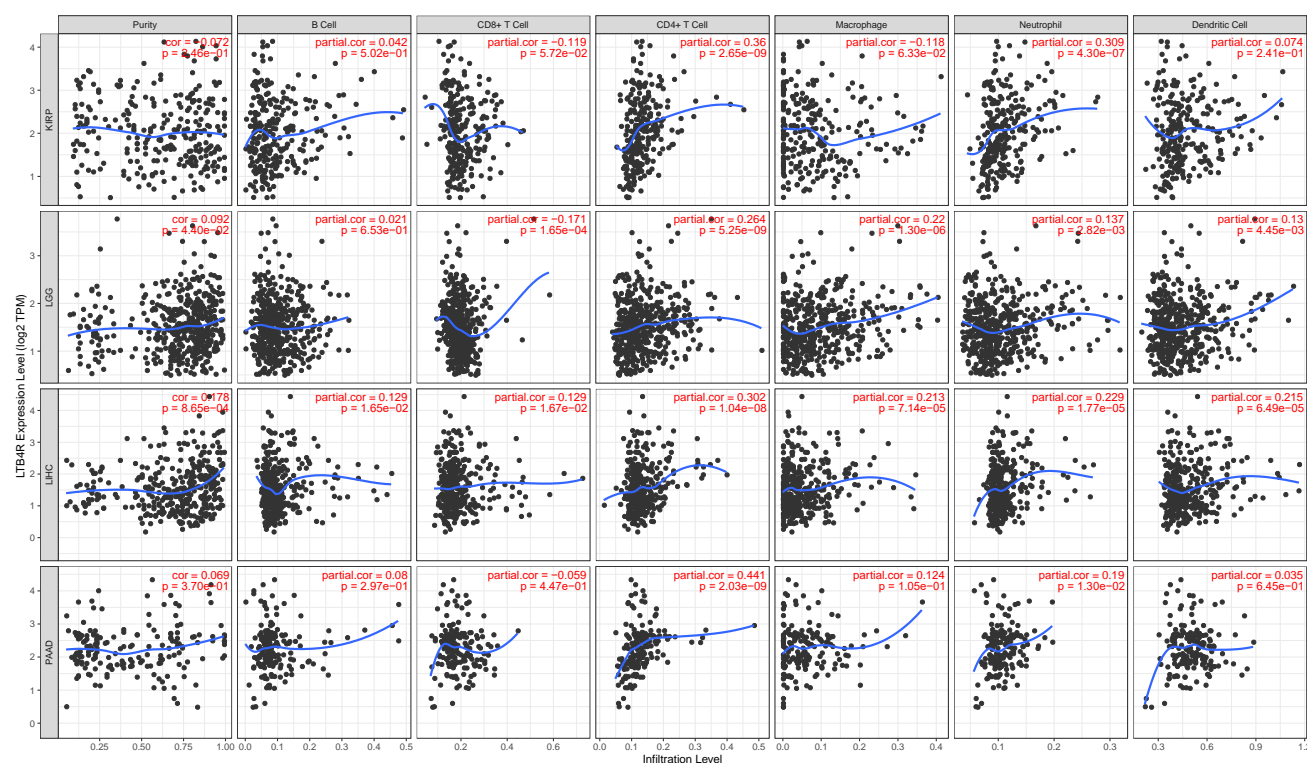

Figure 8. Correlation of LTB4R expression with immune infiltration level in LIHC, PAAD, KIRP and LGG. P < 0.05 is considered as significant.

3.8. Relationships between LTB4R expression and immune markers

We analyzed the connections between LTB4R and many immune cell markers in TIMER to further investigate the potential linkages between LTB4R and invading immune cells. B cells, CD8$^+$ T cells, M1/M2 macrophages, tumor-associated macrophages, monocytes, NK cells, neutrophils, dendritic cells, and regulatory B cells were all identified using these markers. We also analyzed various subtypes of T cells, including follicular helper T, Th1, Th2, Th9, Th17, Th22, regulatory T cells, and exhausted T cells, etc.

After adjusting for tumor purity, LTB4R expression was found to be strongly linked with 16 of 38 immune cell markers in PAAD and 27 of 38 immune cell markers in LIHC in TIMER, 13 of 38 immune cell markers in KIRP and 24 of 38 immune cell markers in LGG (Table 3). As with the above results, LIHC has an abundant type of immune cell infiltration, which may be associated with its good prognosis. Additionally, the relationships of LTB4R with B cell, TF, Treg cell and Macrophage cell, were partially different in LGG, PAAD and KIRP. For example, high expression of LTB4R in LGG and LIHC correlated significantly with Treg cells, while no significant association was seen in KIRP and PAAD. Hence, these results confirm our speculation that LTB4R expression in pan-cancer correlates with immune cell infiltration in different manners, which can help explain the differences in patient survival.

Table 3. Correlations between LTB4R and gene markers of immune cells in LIHC, PAAD, KIRP and LGG.

Gene type	Gene marker	Adjustment by tumor purity							
		LIHC		PAAD		KIRP		LGG	
		Cor	P	Cor	P	Cor	P	Cor	P
B cell	CD19	0.185	**	0.144	6.09e-02	0.002	9.69e-01	0.174	**
	CD38	0.22	***	0.123	1.08e-01	0.081	1.92E-01	0.007	8.85e-01
CD8+ T cell	CD8A	0.205	**	0.219	*	0.147	1.82e-02	0.045	3.25e-01
	CD8B	0.138	1.03E-02	0.136	7.61e-02	0.102	1.01e-01	0.023	6.22e-01
TF	CXCR5	0.206	**	0.194	1.08e-02	0.169	*	0.318	***
	ICOS	0.19	**	0.156	4.19e-02	0.144	2.08e-02	0.249	***
	BCL6	0.245	***	0.259	**	0.324	***	0.253	***
Th1	IL12RB2	0.285	***	-0.084	2.77e-01	0.17	*	-0.094	4.10e-02
	IL27RA	0.221	***	0.324	***	0.195	*	0.066	1.52e-01
Th2	CCR3	0.087	1.06E-01	0.024	7.57e-01	0.28	***	0.131	*
	STAT6	0.211	***	0.264	**	0.201	*	0.088	5.43e-02
	GATA3	0.261	***	0.097	2.05e-01	0.076	2.21e-01	0.206	***
Th9	TGFBR2	0.117	3.01E-02	0.067	3.86e-01	0.012	8.47e-01	0.045	3.26e-01
	IRF4	0.207	**	0.165	3.05e-02	0.084	1.80e-01	0.192	***
Th17	STAT3	0.163	*	0.199	*	0.069	2.69e-01	0.184	***
	RORC	0.197	**	0.113	1.42e-01	-0.113	6.92e-02	0.081	7.52e-02
Th22	CCR10	0.311	***	0.419	***	0.316	***	0.503	***
	AHR	0.03	5.79E-01	0.076	2.25e-01	0.313	***	0.132	*
Treg	FOXP3	0.313	***	0.202	*	0.108	8.32e-02	0.295	***
	CCR8	0.221	***	0.056	4.68e-01	-0.06	3.39e-01	0.278	***
T cell exhaustion	CTLA4	0.152	*	0.252	**	0.236	**	0.245	***

Gene type	Gene marker	Adjustment by tumor purity							
		LIHC		PAAD		KIRP		LGG	
		Cor	P	Cor	P	Cor	P	Cor	P
	LAG3	0.221	***	0.317	***	0.278	***	0.475	***
Macrophage	CD68	0.131	1.51E-02	0.124	1.07e-01	-0.027	6.66e-01	0.196	***
M1	NOS2	0.156	*	0.12	1.18e-01	0.001	9.93e-01	-0.031	4.99e-01
M2	ARG1	0.033	5.37E-01	0.226	*	0.141	2.38e-02	-0.003	9.24e-01
	CD163	0.234	***	0.084	2.73e-01	-0.08	2.02e-01	0.189	***
TAM	HLA-G	0.158	*	0.097	2.05e-01	-0.075	2.29e-01	0.18	***
	CD80	0.235	***	0.241	***	0.07	2.63e-01	0.124	1.06e-01
	CD86	0.227	***	0.078	3.09e-01	-0.046	4.62e-01	0.129	*
Monocyte	CD14	0.083	1.24E-01	0.171	2.55e-02	0.004	9.51e-01	0.131	*
NK	XCL1	0.156	*	0.166	3.02e-02	0.155	1.28e-02	0.166	**
	KIR3DL1	0.22	***	0.005	9.43e-01	0.137	2.78e-02	-0.002	9.69e-01
	CD7	0.117	2.94E-02	0.315	***	0.28	***	0.224	***
Neutrophil	MPO	0.112	3.83E-02	0.134	8.13e-02	-0.091	1.44e-01	-0.079	8.32e-02
DC	CD1C	0.125	2.05E-02	0.207	*	0.191	*	0.098	3.14e-02
	CLEC9A	0.084	1.21E-01	0.204	*	0.054	3.89e-01	-0.171	**
Breg	CD1d	0.298	***	0.292	**	0.364	***	0.318	***
	CD5	0.206	**	0.221	*	0.082	1.90e-01	0.166	**

Tfh, follicular helper T cell; Th, T helper cell; Treg, regulatory T cell; TAM, tumor-associated- macrophage; NK, natural killer cell; DC, dendritic cell; Breg, regulatory B cell; None, correlation without adjustment; Purity, correlation adjusted for tumor purity; Cor, R value of Spearman's correlation. *P < 0.01; **P < 0.001; ***P < 0.0001.

4. Discussion

LTB4 is produced from arachidonic acid (AA) through the sequential actions of 5-Lipoxygenase (5-LO), 5-lipoxygenase-activating protein (FLAP), and Leukotriene A4 hydrolase (LTA4H). LTB4's biological actions are mediated by G-protein coupled receptors, BLT1 (LTB4R with high affinity) and BLT2 (low affinity). The disparities in results across tumor models and human research raise numerous critical issues about the role of the leukotriene B4 pathway in cancer, including pancreas, colon, stomach, prostate, ovaries, and lungs cancers [25]. LTB4R receptor activation involves signaling pathways such as cAMP, PLC/IP3/Ca2+/PKC [26], and increases the production of the downstream adaptor protein MyD88 as well as MyD88-dependent NF-KB activation [27].

Although the association between LTB4R expression and tumor proliferation and development has been verified at the molecular level and in animal models, extensive research for clinical diagnosis and treatment has not been undertaken [28,29]. As past research and recent findings suggest, LTB4R may play distinct roles in different situations [30,31], necessitating further investigation into the links between LTB4R and various types of cancer.

Using separate datasets in Oncomine and PrognoScan, as well as TCGA data in GEPIA and TIMER, this work investigated the expression levels of LTB4R and depicted the prognostic landscape on a pan-cancer basis. Based on the results of these four databases, we found that LTB4R was advantageous in PAAD, OC, UVM, and LIHC but was detrimental in KIRP, KIRC, AML, and LGG. LTB4R expression levels in PRAD and THCA were much lower than in normal tissues, while KIRP, LIHC, and READ expression levels were significantly higher. By the way, we initially concluded from the HCMDB database that single gene (LTB4R) is of little value in identifying metastatic tumors. In this study, we also discovered that the prognosis of LTB4R and cancer may fluctuate depending on factors such as gender, race, and tumor stage. More in-depth and detailed research will be required in the future to find a plausible explanation for these disparities.

Through relevant gene network maps and functional enrichment analysis, we speculate that the role of LTB4R may be different in pan-cancer. For example, LTB4R may be involved in LGG pathogenesis through the glioblastoma signaling pathway. While based on the gene characteristics and functional enrichment results, we speculate that LTB4R largely influences the development of multiple tumors through inflammation and immune microenvironment. However, it is unknown whether biological responses triggered by this receptor result in tumor-promoting inflammation or anticancer immunity. Understanding the TME, including immune cell infiltration, can help identify crucial pathways driving tumor formation. Our findings demonstrate that LTB4R expression has significant relationships with the infiltration level of immune cells in LIHC; while in KIRP, LGG, and PAAD, LTB4R was correlated with the level of immune cell infiltrations such as CD4+T cells and neutrophils (Figure 8). Even though a cause-effect relationship could not be established in the current study. As expected, the connections between LTB4R expression and immune cell markers like CD19, CD38, and MPO are not always the same as the overall trend, implying that particular interactions between LTB4R and distinct immune cell subtypes exist (Table 3). Interestingly, the degree of LTB4R expression in PAAD is not connected to tumor purity with a positive prognosis, implying that it is expressed equally in tumor cells and the tumor microenvironment. However, in LIHC and LGG, LTB4R expression was found to have a substantial positive connection with tumor purity, indicating that it is more abundant in tumor cells. B cells, macrophages, and dendritic cells were the antigen-presenting cells that substantially correlated with LTB4R expression in LIHC; however, in KIRP,

PAAD, they had no association to LTB4R (Figure 8). These discrepancies suggest that cancers differ in their ability to recruit antigen-presenting cells to the TME.

LTB4R has already been linked to cancer prognosis in studies. Several investigations have demonstrated that LTB4R appears to promote cancer progression primarily through immunological regulation and that LTB4R expression on CD8+T cells is critical in their trafficking to tumors [32,33]. Preclinical and clinical researches have revealed that the presence of tumor-infiltrating CD8+T lymphocytes is related with a favorable prognosis and a longer survival advantage [34], and this finding is also reflected in our results (Table 3). The migration of cytotoxic T lymphocytes (CTLs) is a complex process that requires the coordination of numerous entities such as adhesion molecules, chemokines/chemokine receptors, and specific vasculature at the target region. Although LTB4R is required for CTL accumulation during allergic inflammation [35], the anti-tumor effect of LTB4R via CTL migration to tumors has not been studied till recently. In addition, our results indicated that LTB4R has the potential to activate Tregs and induce T cell exhaustion. The increase in LTB4R expression positively correlates with the expression of Treg and T cell exhaustion markers (CTLA4 and LAG3 Table3). Furthermore, significant correlations can be found between LTB4R expression and the regulation of several markers of T helper cells (Th1, Th2, Th17 and Th22). These correlations could be indicative of a potential mechanism where LTB4R regulates T cell functions in LIHC, PAAD, LGG and KIRP. Together these findings suggest that the LTB4R plays an important role in recruitment and regulation of immune infiltrating cells.

Despite the fact that we combined data from several sources, this study had limitations. First, tumor tissue information was used to obtain a major percentage of the microarray and sequencing data. As a result, systematic bias could have been introduced by the cell-level examination of immune cell markers. To address this issue, further research with higher resolution, such as single-cell RNA sequencing, should be conducted [36,37]. Second, because there was only one LIHC dataset with complete clinicopathological features, the results obtained were limited. More clinical data will need to be explored in the future to elucidate the association between LTB4R and cancer patients' clinical features. Third, due to discrepancies amongst databases, we were unable to properly characterize LTB4R as either beneficial or harmful to tumors. Fourth, this work simply did a bioinformatics analysis of LTB4R expression and patient survival across multiple databases, with no in vivo/in vitro trials. Fifth, despite discovering that LTB4R expression correlates with both immune cell infiltration and patient survival in malignancies, we were unable to demonstrate that LTB4R affects patient survival via immune infiltration. Prospective research concentrating on LTB4R expression and immune infiltration in cancer patients could assist provide a conclusive solution in the future.

5. Conclusions

In conclusion, LTB4R has the potential to influence pan-cancer prognosis and to correlate with immune infiltration. LTB4R is associated with various immune cell infiltration patterns in the TME. LTB4R has the potential to be a prognostic biomarker in pan-cancer. These discoveries could lead to an immuno-based anti-tumor therapy that involves altering the energy systems of tumor cells or tumor microenvironment infiltrates.

Disclosure

Sidan Long and Shuangshuang Ji are the first authors.

Acknowledgments

Conflict of interest

The authors declare there is no conflict of interest.

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