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High HBXIP expression is related to poor prognosis in HCC by extensive database interrogation

Z.-Y. GUO[1], L.-P. JIANG[2], Z.-T. ZHU[3]

[1]Department of Radiology, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China
[2]Department of Radiotherapy, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China
[3]Department of Tumor Treatment Center, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China

Abstract. – OBJECTIVE: The involvement of HBXIP in cancer development and cancer cell survival is well known. This work probed the potential of HBXIP as a prognostic biomarker in hepatic cell cancer (HCC).

MATERIALS AND METHODS: First, pan-cancer analysis of HBXIP expression was conducted using The Cancer Genome Atlas (TCGA) database to validate the expression of HBXIP in different cancers. The GSE14520 (GPL3721 Subset) database was used to validate HBXIP in HCC. The association between survival outcomes and prognostic factors was assessed employing univariate and multivariate survival analyses for TCGA Liver Hepatocellular Carcinoma. The biological function of the HBXIP Gene was annotated by gene set enrichment analysis. The relationship between HBXIP expression and immune cells and immune markers was analyzed from the Gene Expression Profiling Interactive Analysis (GEPIA) database.

RESULTS: Malignant tissues demonstrated evident upregulation of HBXIP at transcriptional and protein levels over normal tissues ($p < 0.05$) with this elevated expression linked to an advanced tumor stage in HCC cohorts. Univariate analysis revealed an evident correlation emerged between prognosis and HBXIP for GSE14520 databases ($p < 0.05$). The disease-free survival (DFS) and overall survival (OS) (five-year values) were lower in samples demonstrating elevated HBXIP (HR: 2.413; 95% CI 1.601, 3.638; $p < 0.001$) and (HR: 1.613; 95% CI 1.446, 1.844; $p = 0.003$), respectively *vs*. lower HBXIP expression. HBXIP emerged as an independent factor in OS prognosis (HR 2.184; 95% CI 1.495, 3.196; $p < 0.001$) and DFS (HR 1.764; 95% CI 1.261, 2.466; $p < 0.001$), respectively according to multivariate analysis. Further, multiple Cox analyses in the validation cohort revealed that independent factors for OS were HBXIP, AJCC T stage, vascular invasion, and tumor status with the C-index score of 0.727 (95% CI, 0.704 to 0.750). HBXIP level showed a significantly positive association with tumor immune cell infiltration, and biomarkers of immune cells; besides, the rectum Rho GTPase effectors signaling pathway was also identified.

CONCLUSIONS: HCC advancement and survival involves HBXIP, which also emerged as a functional biomarker for HCC survival prediction.

Key Words:
Biomarker, Hepatic cell cancer, HBXIP, Prognosis.

Introduction

Hepatocellular carcinoma (HCC) accounts for the predominant malignancies. The death rate associated with HCC ranks second in East Asia and the sixth most common in western countries[1,2]. However, the high postoperative recurrence remains a roadblock, with the 5-year cumulative recurrence at 77-100% and the remnant liver demonstrating 80-95% of recurrences with a 5-year survival rate <15%[3]. HCC incidence is speedily increasing globally. The risk factors for the malignancy include alcohol usage, obesity, diabetes, chronic infections of HBV and HCV (hepatitis B virus and hepatitis C virus), and several metabolic diseases[4].

Comprehending the mechanism of HCC occurrence and progression and formulation of approaches to effectively address the malignancy is the need of the hour. Prognosis employing molecular approaches is receiving the attention to pathological balance diagnosis to augment HCC patient lifespan[5,6].

Initially discovered as bound to hepatitis B virus X protein, HBXIP or hepatitis B X-interacting protein is a conserved 19 kDa protein, which is the symbol for human gene LAMTOR5 (Late Endosomal/Lysosomal Adaptor and MAPK And MTOR Activator 5) gene[7]. Its aberrant profile is manifested as malignancy with faulty chromo-

Corresponding Author: ZhiTu Zhu, MD; e-mail: zhu_zhitu@163.com

some segregation and genetic instability due to boosted centrosome production and multipolar mitotic spindles[8]. HBXIP can promote cell growth, proliferation, migration, and angiogenesis with its overexpression demonstrated in many malignancies, such as non-small-cell lung cancer, esophageal squamous cell carcinoma, ovarian cancer, cervical cancer, and breast cancer[8-10]. The activation of the PI3K/Akt/mTOR pathway documented in head and neck squamous cell carcinoma (HNSCC) development is also linked partially to HBXIP-mediated stimulation[11]. The clinical attributes and the survival outcome for ovarian cancer and esophageal squamous cell carcinoma are linked to elevated HBXIP expression[8,12].

Elevated HBXIP expression in many malignancies and its correlation with poor clinical outcomes is being examined by research. HBXIP levels in HNSCC and esophageal squamous cell carcinoma are linked to poorer prognosis and worse survival outcomes in terms of high TNM clinical stage and pathological grade along with metastasis and increased vascular invasion indicative of poor OS and DFS[13,14]. With the dearth of studies exhaustively exploring this protein in HCC studied in clinical databases, this present study probes the functioning of HBXIP in HCC.

This entailed scoring HBXIP expression in the pan-cancer database, whole-genome expression microarray (GEO, Accession Number GSE14520: GPL3721 Subset) followed by its validation in The Cancer Genome Atlas (TCGA-LIHC). Procedures entailing human participants adhered to the requisite stipulations as elaborated further below.

Materials and Methods

Gene Expression Profiling Interactive Analysis (GEPIA) Database Analysis

GEPIA (http://gepia.cancer-pku.cn/) is an online data repository tool for cancer and normal gene-expression profiling analysis based on the UCSC Xena project, including TCGA and the Genotype-Tissue Expression (GTEx) data. HBXIP/LAMTOR5 expression and its prognostic values in various types of human cancer were evaluated by GEPIA. p-value < 0.05 was considered statistically significant.

One Database for the Validation of HBXIP Expression and Overall Survival of HCC

The link between OS of HCC patients and HBXIP mRNA expression was first probed in GSE14520 (GPL3721 Subset) (https://www.ncbi.nlm.nih.gov/geo/) to score the impact of HBXIP mRNA levels and OS. The inclusion criteria for these two databases entailed: (1) HCC diagnosed by pathological examination, (2) no prior chemo/radiotherapy i.e., neoadjuvant therapy, and (3) comprehensive survival information. The clinical end-point to OS was death with a tumor at the last follow-up.

Exploration HBXIP Expression and Survival of HCC from TCGA-LIHC Dataset

TCGA-LIHC was selected as an independent cohort to validate the prognostic significance of HBXIP on OS and DFS in HCC. This database data was downloaded from the UCSC Xena browser (https://xenabrowser.net/). The RNA sequencing data (FPKM) was the source of the HBXIP mRNA expression levels. The clinical information of the TCGA cohort is summarized in I. The study was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University. After excluding the data lacking complete survival information, this work entailed 366 HCC patients showing complete follow-up data.

Immunohistochemistry Data

The Human Protein Atlas (https://www.proteinatlas.org) is a website encompassing expression data of immunohistochemical evaluation of close to twenty ubiquitous malignancies of twelve individual tumor subtypes[15]. This facilitates the ascertainment of expression patterns pertaining to the subtype. The immunohistochemistry images facilitated a direct comparison of HBXIP/LAMTOR5 protein profiles in HCC *vs.* normal human tissues.

Analysis of the TIMER Database

TIMER (https://cistrome.shinyapps.io/timer/) is an online server to analyze the relationship between genes and tumor-infiltrating immune cells. In this study, the correlation between HBXIP/LAMTOR5 expression level and different infiltration levels of immune cells in HCC had been analyzed by TIMER. The significant level was set as p-value <0.05.

Construction of PPI Network

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) was used to investigate the interaction between HBXIP/LAMTOR5 and other

genes. The LAMTOR5-related PPI network with a minimum required interaction score >0.25 and their interactions were analyzed with Cytoscape 3.7.1 (https://cytoscape.org/).

Statistical Analysis

The clinicopathological variable- HBXIP link was scrutinized employing the Pearson χ^2-test with the use of the Fisher exact test when necessary. The date of surgery to the date of local or distant disease relapse was the DFS while the date of diagnosis to the date of mortality, or last follow-up was defined as the OS. Data of those patients were eliminated who lacked events or mortality at the last follow-up. The Kaplan-Meier method was employed for survival curves whose differences were probed by the log-rank test. The HR (hazard ratio) for DFS and OS entailed the use of univariate Cox proportional hazards regressions from where multivariate analysis was done for those deemed significant in the univariate analyses ($p < .05$). The hazard risk of each

Table I. The expression of HBXIP and clinicopathologic features in the TCGA-LIHC cohort.

Characters	Levels	IBXIP High (n=219)	IBXIP Low (n=146)	χ^2	p
Age65	<=65	136	91	.037	.527
	>65	83	55		
SEX	FEMALE	69	50	.001	.539
	MALE	150	96		
RACE	ASIAN	105	50	0.003	.332
	WHITE	93	89		
	OTHER	21	7		
ECOG Performance Score	0	81	69	.478	.480
	1	41	30		
	>=2	23	11		
TUMOE status	TUMOR FREE	146	85	2.503	.243
	With Tumor	58	50		
HEPATISIS	NO	53	40	.119	.286
	YES	166	106		
GRADE	G1	28	27	6.8	0.083
	G2	101	74		
	G3	76	42		
	G4	11	1		
Residual tumor	R0	190	130	.987	.755
	R1	11	7		
AJCC T stage	T1&T2	166	105	.448	.503
	T3&T4	52	39		
AJCC N stage	N0	151	97	.569	.194
	N1	4	0		
AJCC M stage	M0	165	98	.679	.204
	M1	2	1		
Tumor stage	Stage I	93	77	9.455	0.049
	Stage II	62	22		
	Stage III	48	35		
	Stage IV	3	1		
Vascular invasion	No	116	89	3.245	.206
	Yes	71	35		
ChildPugh stage	A	131	85		.955
	B	13	9		
AFP	<=200	131	89	.079	.96
	>200	25	17		
Fibrosis	No	33	41	9.072	0.010
	Yes	87	48		
Death	No	132	103	5.390	0.028
	Yes	87	43		
Recurrent	No	80	61	4.534	0.071
	Yes	101	72		

Note: ECOG Eastern Cooperative Oncology Group; AFP alpha-fetoprotein; AJCC American Joint Committee on Cancer

factor was scored considering the HR at 95% CI. All reported *p*-values were two-sided with significance deemed at 0.05. IBM SPSS software was employed for all analyses.

Results

Pan-Cancer Analysis of LAMTOR5 Expression

To explore the possible roles of LAMTOR5 in carcinogenesis, we first analyzed its expression in 20 types of human cancers in comparison with normal samples, as shown in Figure 1A. LAMTOR5 was significantly upregulated in the following 12 cancer types, including bladder carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), colon adenocarcinoma (COAD), glioblastoma multiforme (GBM), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), uterine carcinosarcoma (UCEC), and thyroid cancer (THCA); downregulated in two cancer types, involving kidney chromophobe (KICH) and kidney renal clear cell carcinoma (KIRC); without significant differences in six cancer types, involving cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), head and neck squamous cell carcinoma (HNSC), kidney renal papillary cell carcinoma (KIRP), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG) or rectum adenocarcinoma (READ). Next, we further validated the expression of LAMTOR5 in these 20 cancer types using the GEPIA database. As presented in Figures 1 B and E, in comparison with corresponding normal controls, LAMTOR5 expression in CHOL, GBM, LIHC, and PAAD increased statistically, and decreased in KICH (Figure 1F). Taken together, LAMTOR5 was upregulated in CHOL, GBM, LIHC, PAAD, and downregulated in KICH, indicating that LAMTOR5 may function as a crucial regulator in the carcinogenesis of some types of cancer.

The Prognostic Values of LAMTOR5 Gene in Human Cancer

Next, survival for LAMTOR5 in pan-cancer was analyzed. Two prognostic indices, overall survival (OS) and disease-free survival (RFS), were included. For OS, high expression of LAMTOR5 in HCC had an unfavorable prognosis, but KIRC patients with higher expression of LAMTOR5 indicated better prognosis (Figure 2 A, B, C). For RFS, among all cancer types, only increased expression of LAMTOR5 indicated poor prognosis in HCC, while STAD and KIRC patients with higher expression of LAMTOR5 indicated better prognosis (Figure 2 D, E, F). No statistical significance of LAMTOR5 in predicting patient prognosis in other cancer types was observed. The combination of OS and RFS, LAMTOR5 may be utilized as an unfavorable prognostic biomarker in patients with HCC.

In Silico HBXIP mRNA Expression Patterns in HCC

HBXIP is a conserved 19-kD protein, which is encoded by four exons on chromosome 1p13.3 (1: 110401253-110407924; NCBI gene 10,542). HBXIP is also known as late endosomal/lysosomal adaptor, MAPK, and MTOR activator 5 (LAMTOR5)[7]. A remarkable rise in HBXIP mRNA was demonstrated in 217 malignant samples over healthy tissues (GSE14520) ($p < .001$; Figure 3A). HBXIP mRNA expression demonstrated a copious variation from stage I to stage III with stages II and III documenting the most significant difference ($p < 0.001$; Figure 3B). This is indicative of the impact of HBXIP on HCC initiation and progression. For the GSE14520 database**,** HBXIP mRNA expression was categorized into low or high-expression subgroups employing the median HBXIP mRNA expression. HBXIP could predict OS in GSE14520 (HR 1.48; 95% CI 0.95-2.28; $p < .001$) (Figure 3C).

HBXIP Expression and Clinicopathological Features

The next step following the HBXIP mRNA expression profile in HCC was its protein expression profiling employing the Human Protein Atlas. HCC samples demonstrated elevated HBXIP protein expression (Figure 4A), while normal tissues documented low protein expression (Figure 4B). The results thus demonstrate overexpressed HBXIP protein in HCC patients.

HBXIP Overexpression is Predictive of Poor Prognosis in TCGA-LIHC (Validation Cohort)

HBXIP overexpression and poor OS and DFS

Of the 355 cases, 129 patients (36.34%) died due to the malignancy during the follow-up (median

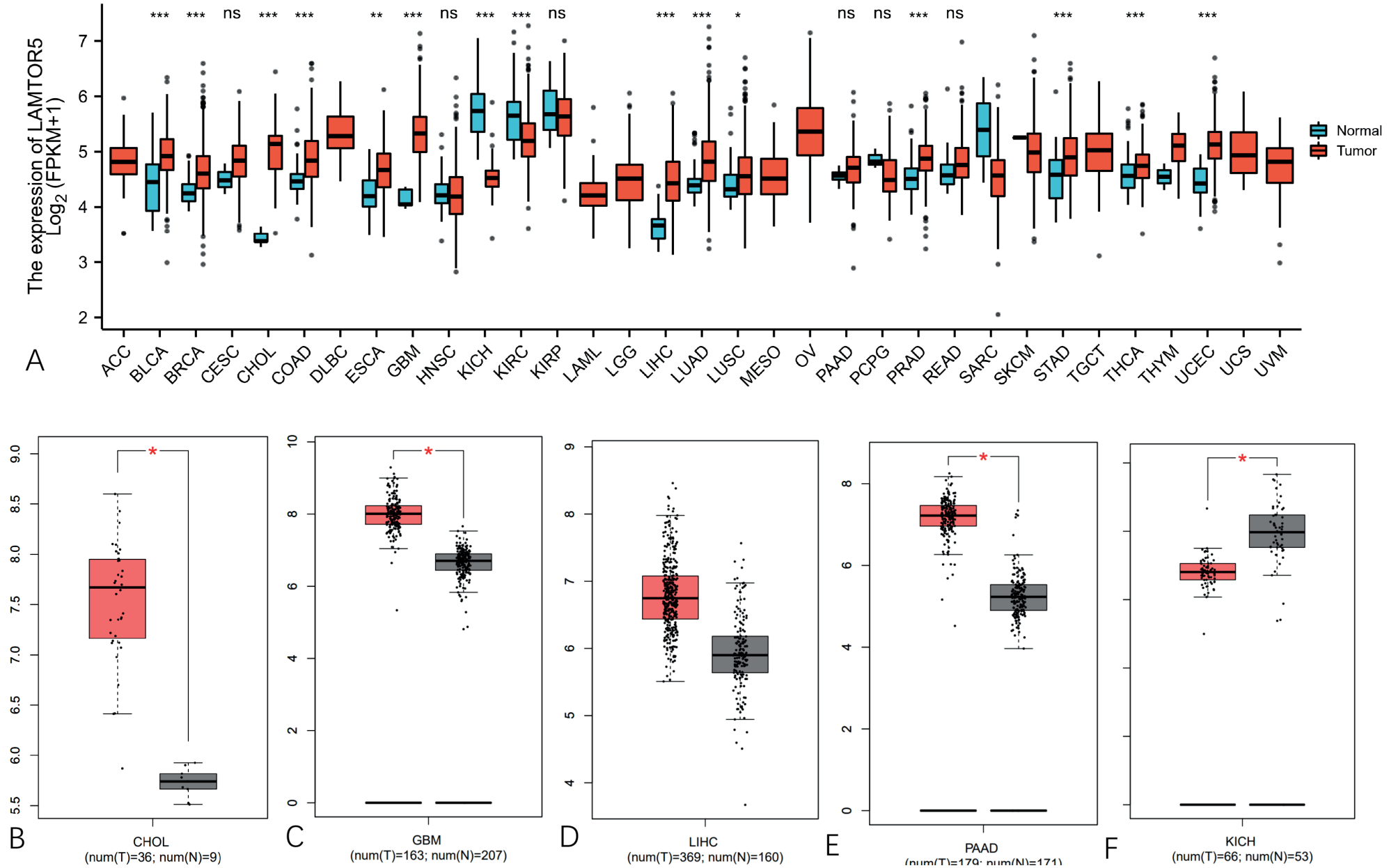

Figure 1. HBXIP expression analysis in multiple cancers. **A,** The expression of HBXIP in 20 types of human cancers based on TCGA cancer and normal data. **B-F,** HBXIP expression in TCGA. CHOL (**B**), GBM (**C**), LIHC (**D**), PAAD (**E**), KICH (**F**) tissues compared with corresponding TCGA and GTEx normal tissues. **p*-value < 0.05; ***p*-value < 0.01; ****p*-value < 0.001.

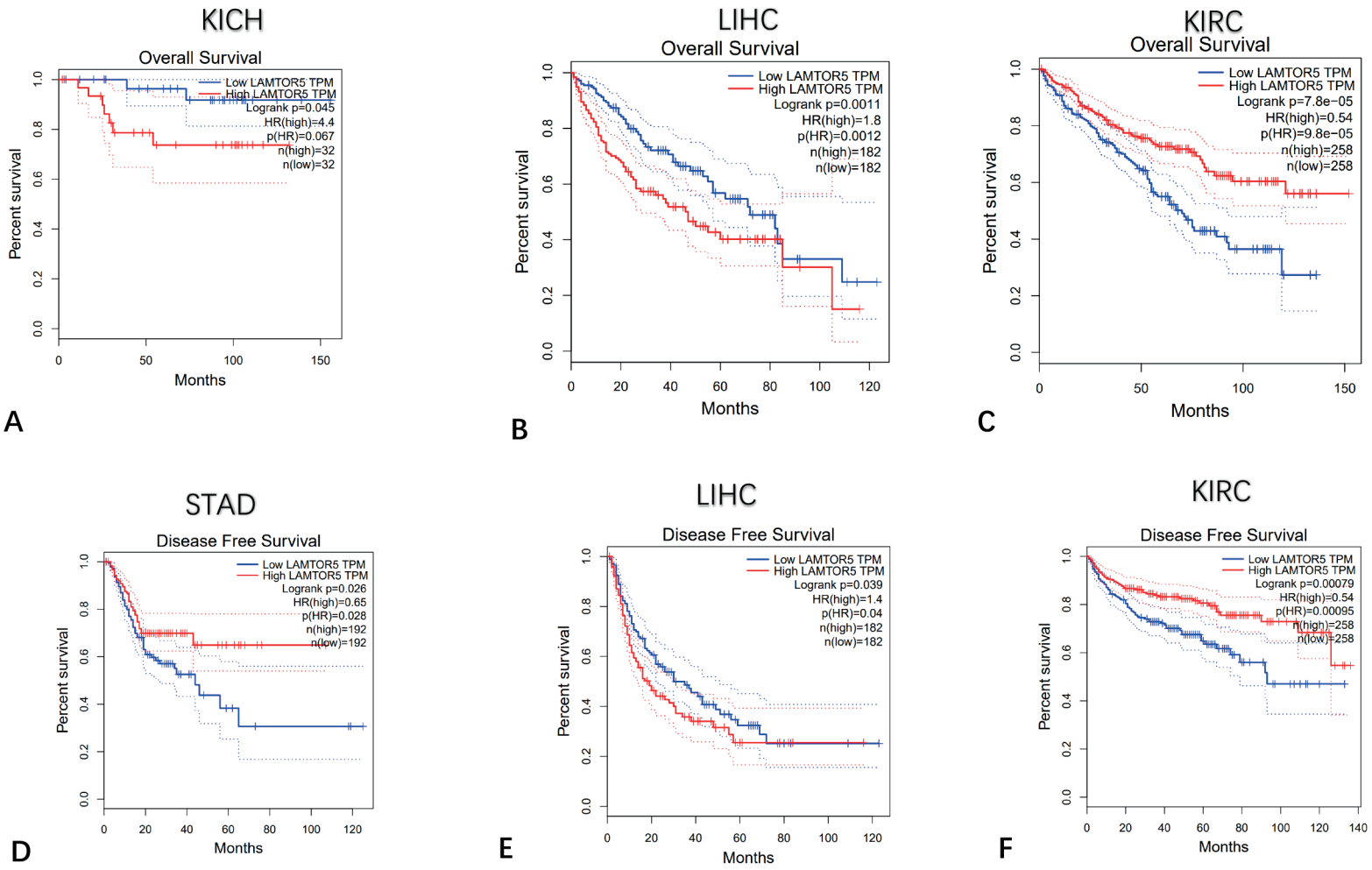

Figure 2. The overall survival (OS) and disease-free survival (DFS) analysis for LAMTOR5 in various human cancers determined by the analysis of the GEPIA database: (**A-C**) The OS plot of LAMTOR5 in KICH (**A**), LIHC (**B**), KIRC (**C**); (**D-F**) The DFS plot of LAMTOR5 in STAD (**D**), LIHC (**E**), and KIRC (**F**).

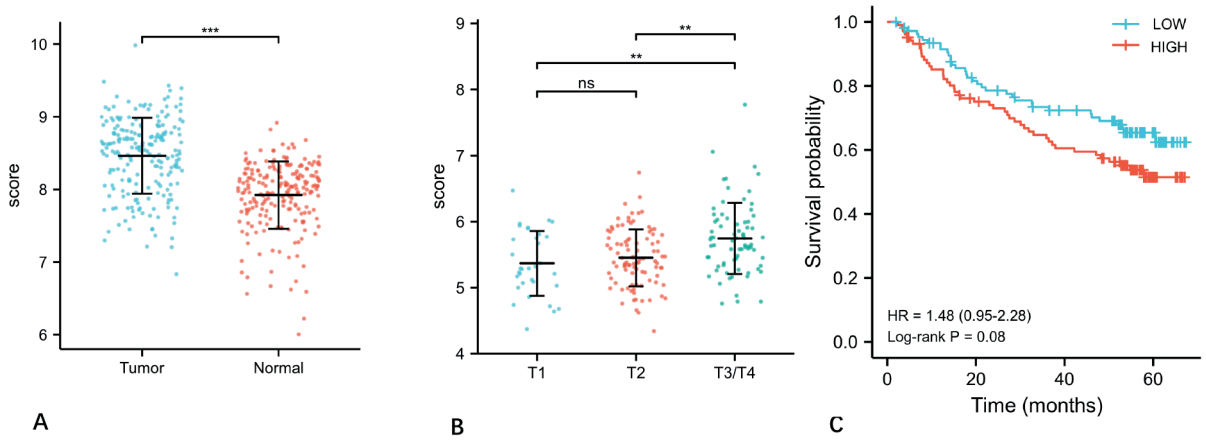

Figure 3. Bioinformatics-based HBXIP mRNA expression analyses in HCC from GSE14520 database. **A,** 217 paired samples of HCC and normal control demonstrating copious elevation of higher HBXIP mRNA in malignant samples over normal liver tissues ($p < .001$). **B,** Stage I to stage III HCC documenting a gradual rise in HBXIP in GSE14520 ($p < .001$). **C,** High expression of HBXIP indicating poor overall survival.

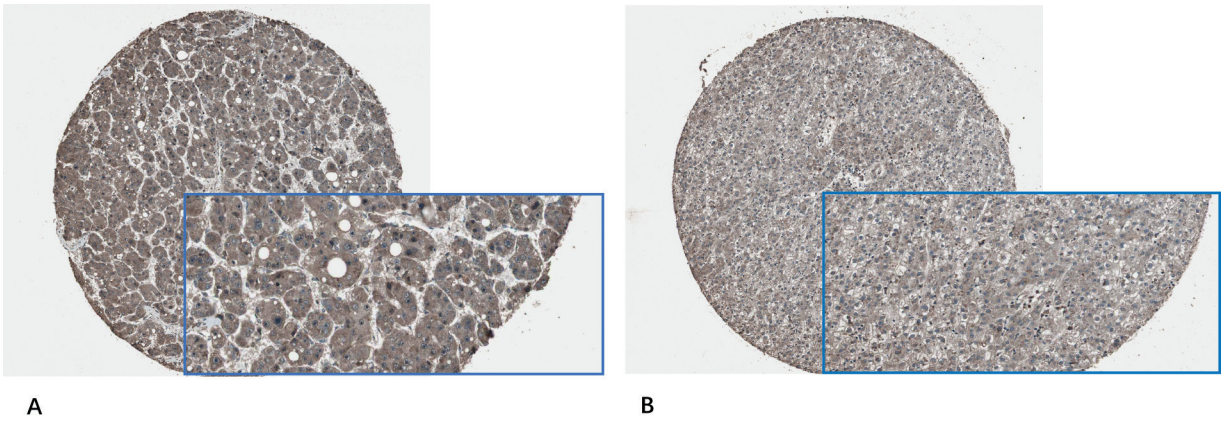

Figure 4. Illustrative immunohistochemistry images of HBXIP expression in HCC and normal liver tissues (Human Protein Atlas) demonstrating higher expression levels of HBXIP in the hepatic cancer tissues (**A**) and low levels in the normal liver tissues (**B**).

survival time 56.17 ± 8.69, 95% CI: 39.13-73.20). Patients were categorized as the high HBXIP group (90/219) and the low expression (39/136) group. The clinicopathologic characteristics of the patients are listed in detail in Table I. The ECOG (Eastern Cooperative Oncology Group) Scale of Performance Status (PS) did not show a significant difference between the two groups.

The median survival time in the high HBXIP expression (46.2 ± 7.03, 95% CI: 32.42-59.98) demonstrated a conspicuously shorter OS vs. those with low expression (81.87 ± 11.45, 95% CI: 59.43-104.30), log-rank $p < .001$).

Of the 307 cases, 141 patients (54.1%) demonstrated tumor recurrence during the follow-up. This recurrence was more in the high expression HBXIP group (87/189) than in the low expression (54/118) group. The median DFS in the high HBXIP expression group (21.87 ± 4.60, 95% CI 12.85-30.88) demonstrated a shorter DFS over those with low expression (40.97 ± 8.83, 95% CI (23.66-58.27), log-rank $p = .074$).

Analysis for overall survival

OS emerged to be linked with (all at 95% CI) HBXIP (HBXIP high *vs.* Low, HR 2.413; 95% CI

Table II. Univariate and multivariate Cox proportional hazard analyses of HBXIP expression and overall survival for patients with HCC in the TCGA-LIHC cohort.

Characters	Univariate analysis HR(95% CI)	p	Multivariate analysis HR(95% CI)	p
Sex (female/male)	1.206 (0.798,1.823)	0.374		
Age(<=65/>65)	0.914 (0.616,1.355)	0.653		
Tumor stage	0.735 (0.204,2.646)	0.690		
T stage	0.832 (0.074,9.330)	**0.026**	1.076 (0.144, 8.046)	**0.001**
Vascular invasion	0.666 (0.383,1.159)	0.164	0.502 (0.316, 0.798)	**0.004**
Tumor Status	0.357 (0.176,0.723)	**0.001**	0.416 (0.215, 0.806)	**0.009**
AFP	0.539 (0.328,0.887)	**0.043**	0.536 (0.353, 0.814)	**0.003**
HBXIP	2.413 (1.601,3.638)	**0.001**	2.184 (1.495, 3.192)	**0.000**

Abbreviations: CI, confidence interval; HR, hazard ratio; AFP alpha-fetoprotein.

1.601-3.638; $p = .001$), tumor stage (T1 & T2 *vs.* T3 & T4, HR 0.832; 95% CI 0.074-9.330; $p = .026$), AFP (AFP $\leq$ = 200 *vs.* AFP > 200, HR 0.539; 95% CI 0.328-0.887; $p = .043$), tumor status (tumor free *vs.* with tumor, HR 1.127; 95% CI 1.081-1.175; $p < .001$). All 355 patients displayed an association of OS with HBXIP, pathologic T stage, tumor status (with tumor or tumor free) and AFP ($p \leq 0.05$). These results are listed in Table II.

OS demonstrated an association with HBXIP (HR 2.184; 95% CI 1.495-3.192; $p <0.001$), tumor stage (T1 & T2 VS. T3 & T4, HR 1.076; 95% CI 0.144-8.046; $p = .001$), vascular invasion (no invasion *vs.* with invasiaon, HR 0.502; 95% CI 0.316-0.798; $p = .004$), tumor status (tumor free *vs.* with tumor, HR 0.416; 95% CI 0.215-0.806; $p = .009$), AFP (AFP $\leq$ 200 *vs.* AFP >200, HR 0.536; 95% CI 0.353-0.814; $p = .003$) as independent prognostic factors. Multivariate analysis revealed that HBXIP, adjacent tissue inflammation, tumor status (with tumor or tumor free), surgery method, and hepatitis occurence were independent predictors of OS ($p < 0.05$; Table II). The c-index was 0.727 (95% CI, 0.75-0.704).

Survival analysis for DFS

DFS was found to be linked with (all at 95% CI) HBXIP (HBXIP high *vs.* Low, HR=1.613; 95% CI 1.446-1.844; $p = .003$), tumor stage (T1 & T2 *vs.* T3 & T4, HR 1.190; 95% CI 1.025-1.381; $p = .023$), AFP (AFP $\leq$ 200 *vs.* AFP > 200, HR 0.539; 95% CI 0.328-0.887; $p = .043$) , tumor status (tumor free *vs.* with tumor, HR 1.106; 95% CI 1.033-1.183; $p = .004$), vascular invasion (no invasion *vs.* with invasiaon, HR 1.054; 95% CI 1.004-1.106; $p = .034$). All 301 patients displayed an association of DFS with HBXIP, pathologic T-stage, vascular invasion, tumor status and AFP ($p \leq 0.05$). These results are listed in Table III.

DFS demonstrated an association with HBXIP (HR 1.764; 95% CI 1.261-2.466; $p = 0.001$), tumor stage (T1 & T2 *vs.* T3 & T4, HR 0.116; 95% CI

Table III. Univariate and multivariate Cox proportional hazard analysis of HBXIP expression and disease-free survival for patients with HCC in the TCGA-LIHC cohort.

Characters	Univariate analysis HR(95% CI)	p	Multivariate analysis HR(95% CI)	p
Sex	0.958 (0.683,1.342)	0.802		
Age	0.767 (0.545,1.079)	0.127		
Hepatitis	1.253 (0.830,1.893)	0.283	0.531 (0.341,0.829)	**0.005**
Fibrosis	0.995 (0.952,1.039)	0.820	0.597 (0.374,0.953)	**0.031**
Tumor Stage	1.067 (0.989,1.151)	0.095		
T stage	1.190 (1.025,1.381)	**0.023**	0.116 (0.24,0.553)	**0.007**
Vascular invasion	1.054 (1.004,1.106)	**0.034**		
Tumor Status	1.106 (1.033,1.183)	**0.004**	0.280 (0.130,0.602)	**0.001**
AFP	0.539 (0.328,0.887)	**0.043**	0.573 (0.369,0.890)	**0.013**
HBXIP	1.613 (1.446,1.844)	**0.003**	1.764 (1.261,2.466)	**0.001**

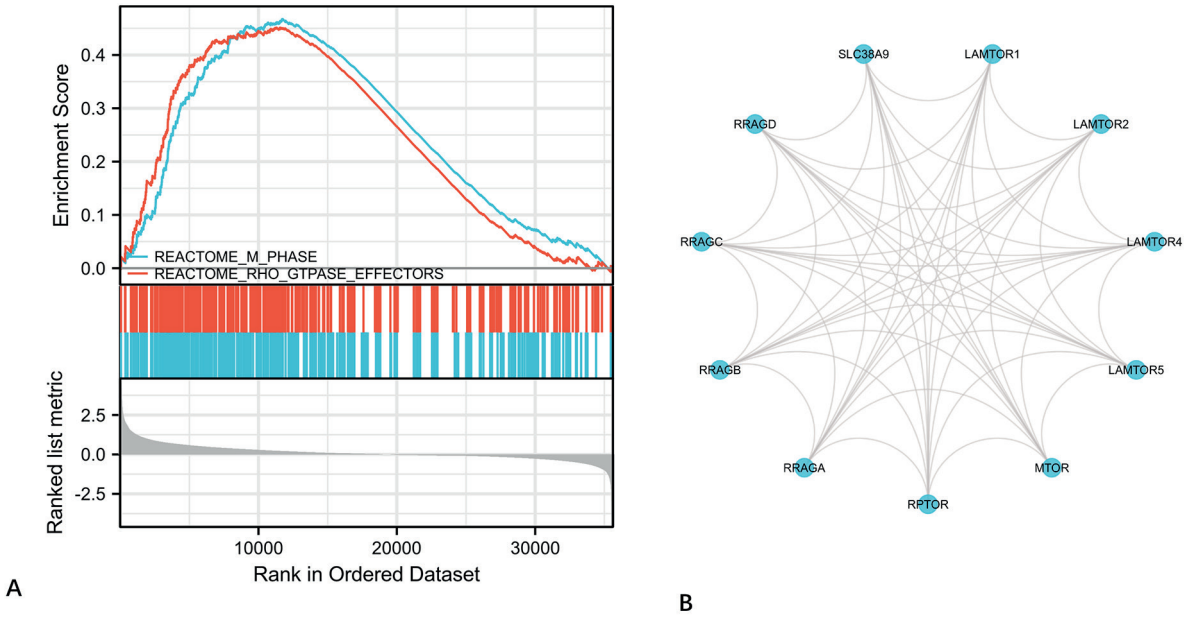

Figure 5. Enrichment plots from gene set enrichment analysis (GSEA) and PPI network of LAMTOR5. **A,** Results of GSEA showed that signaling pathway was differentially enriched in high LAMTOR5 expression phenotype. **B,** PPI network of LAMTOR5 suggested that LAMTOR5 had a close relationship with RRAG-mTOR signaling; ES, enrichment score; NES, normalized ES; FDR, false discovery rate; NOM *p*-value, normalized *p*-value; RRAG, Rag A-D family of GTPases (known as RRAGA, B, C, and D); PPI, protein-protein interaction.

0.24-0.553; p = .007), tumor status (tumor free *vs.* with tumor, HR 0.28; 95% CI 0.130-0.602; p = .001), AFP (AFP<= 200 *vs.* AFP > 2 00, HR 0.573; 95% CI 0.369-0.890; p = .0013) as independent prognostic factors. Multivariate analysis revealed that HBXIP, adjacent tissue inflammation, tumor status (with tumor or tumor free), surgery method, hepatitis occurence were independent predictors of OS ($p < 0.05$; Table III). The c-index is 0.692 (95% CI, 0.665-0.719).

HBXIP/LAMTOR5-Related Signaling Pathway Analysis Performed on GSEA

GSEA was used to screen significantly different signal pathways between high and low expressions of LAMTOR5. p-value < 0.05 was set as significant difference in the enrichment of MSigDB collection (c2.cp.kegg.v7.0.symbols). Signaling pathways that were prominently enriched in high LAMTOR5 expression phenotype were revealed and included the Rag family of small GTPases (RRAG)-mammalian target of rapamycin (mTOR) signaling pathway (Figure 5 A, B).

HBXIP/LAMTOR5 Positively Correlates with Immune Cell Infiltration in HCC

LAMTOR5, a member of the LAMTOR family, is reported to play a critical role in the signal pathways of the mTOR family. In this study, the immune cell infiltration level was significantly different between high and low expressions of LAMTOR5 in HCC. Correlation between the levels of LAMTOR5 expression and immune cell infiltration was also evaluated. LAMTOR5 expression was significantly positively associated with the infiltration of $CD4^+$ T cell, macrophage, neutrophil, and dendritic cell in HCC (Figure 6), and with biomarkers of the infiltrated immune cells, Table IV. These findings indicated that tumor immune infiltration might partially account for LAMTOR5-mediated oncogenic roles in HCC.

Discussion

Studies are unearthing the crucial involvement of HBXIP proteins in the oncogenesis of a slew of malignancies. Although some studies have indicated the close link between HBXIP and HCC patient prognosis and survival, these are small-scale studies that warrant validation in a large clinical cohort. The relevance of this protein in HCC survival prediction has been scrutinized in few reports[16].

In this study, a higher HBXIP mRNA profile emerged for malignant tissues in 215 paired HCC

Table IV. Correlation analysis between LAMTOR5 and biomarkers of immune cells in hepatic cell cancer.

Immune cell	Biomarker	R	*p*
B cell	CD19	0.066	0.21
	CD79A	-0.023	0.66
CD8+ T cell	CD8A	0.058	0.27
	CD8B	0.067	0.2
CD4+ T cell	CD4	0.1	**0.047**
	NOS2	-0.014	0.78
M1 macrophage	IRF5	0.27	**<0.001**
	PTGS2	0.022	0.67
	CD163	0.18	**<0.001**
M2 macrophage	VSIG4	0.15	**<0.001**
	MS4A4A	0.2	**<0.001**
	CEACAM8	0.0097	0.85
Neutrophil	ITGAM	0.34	**<0.001**
	CCR7	-0.029	0.58
	HLA-DPB1	0.21	**<0.001**
	HLA-DQB1	0.12	0.021
Dendritic cell	HLA-DRA	0.18	**<0.001**
	HLA-DPA1	0.18	**<0.001**
	CD1C	0.043	0.41
	NRP1	0.42	**<0.001**
	ITGAX	0.14	**0.007**

and normal tissues from the GSE14520 (GPL3721 Subset) database as opposed to the healthy ones (p<0.001). This trend was on the similar lines for the HBXIP protein with increased expression in HCC over the normal, healthy counterparts in the validation cohort (TCGA-LIHC).

Likewise, stage I to stage III malignancies demonstrated a conspicuous and gradual increase in the HBXIP mRNA expression in the ICGC database. This elevated HBXIP expression was associated with advanced T stage, pathology stage, histology stage, and vascular invasion (p = 0.04) in the validation cohort as shown in Table I. This suggests the critical involvement of HBXIP in HCC oncogenesis. The HBXIP protein profile too was along similar lines with high HBXIP prognostic of an unfavorable 5-year survival for HCC vS. that of low level (38% *vs.* 61%).

This was corroborated in public databases with HBXIP emerging as a predictor of OS in the ICGC and GSE14520 and for RFS only in the ICGC. Subsequent validation in the TCGA database revealed lesser OS and DFS with elevated HBXIP expression. Multivariate analysis corroborated with the utility of HBXIP as an independent prognostic factor for OS. Correlations have been demonstrated for elevated HBXIP expression with tumor metastasis, invasion, and poor prognosis. Several malignancies inclusive of breast cancer[10], cervical carcinoma[17], gastric cancer[18], ovarian carcinoma[19], and colorectal carcinoma[20] have documented high HBXIP expression.

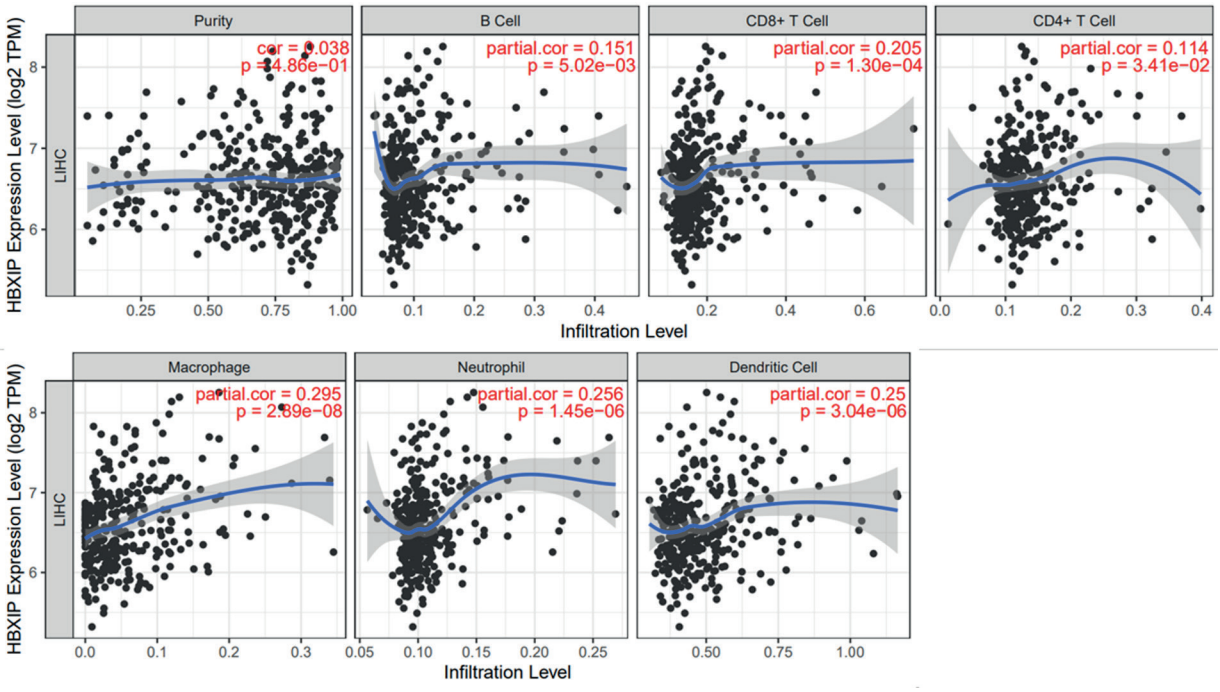

Figure 6. The relationship of immune cell infiltration with LAMTOR5 level in hepatocellular carcinoma (HCC). The infiltration level of various immune cells under different expressions of LAMTOR5 in HCC. **A,** The correlation of LAMTOR5 expression level with tumor purity. **B-G,** The correlation of the level of LAMTOR5 expression with B cell (**B**), CD8+ T cell (**C**), CD4+ T cell (**D**), macrophage (**E**), neutrophil (**F**), or dendritic cell (**G**) infiltration level in HCC.

This overexpression has been linked to poor clinicopathological factors to emerge suggestive of the oncogenic functioning of HBXIP. This led us to propose that HBXIP may be a biomarker in HCC.

A range of complex molecular mechanisms are seen for oncogenesis and HBXIP overexpression: (1) Inhibition of the cytC-caspase mitochondrial apoptotic pathway to constitute HBXIP-survivin complexes and induce resistance in the tumor cells[21]; (2) HBXIP-survivin complex interactions or enhanced HBXIP promoter functioning to augment mitosis and cell division[15]; (3) HBXIP and HBx localization to microtubules and centrosomes, which are vitally involved in tumorigenic cell division[22]; (4) Regulated glucose and lipid metabolism augmenting malignant cell survival; (5) Induction of a regulatory cascade to impact a slew of signaling pathways and miRNAs to augment carcinogenesis.

This work entailed downloading the RNA sequencing data for mRNA expression of HCC patients from the open-access TCGA database encompassing many cancer patient sample sets[23]. Subsequent statistical analyses led to the formulation of a prognostic model for HCC employing HBXIP expression. The survival of HCC patients was diminished when the HBXIP levels were augmented over those with low-risk scores. Following adjustment in multivariate Cox regression analyses, HBXIP levels along with age (in total) and the pathological stage emerged as independent predictors of HCC patient survival. Subsequent stratification analyses demonstrated the relevance and robustness of this HBXIP signature in patient subgroups categorized by age and the pathological stage.

Tumor immune cell infiltration could significantly influence the prognosis of HCC patients[24]. Our result suggested that LAMTOR5 is significantly positively correlated with CD8+ T cell, CD4+ T cell, macrophage, neutrophil, and dendritic cell in HCC. Additionally, LAMTOR5 was also found to be positively associated with biomarkers of these infiltrated immune cells. Our findings indicate that the oncogenic role of LAMTOR5 in HCC could be partially related to the effect of tumor immune infiltration.

Conclusions

Overall, this study reports HCC patient prognosis prediction by an HBXIP signature. The reproducibility and robustness of the signature in another large-scale independent HCC cohort demonstrated its efficacy and importance. Further, the independence of HBXIP expression from clinicopathological variables in prognosis was also documented. The study shows an improved prediction of HCC patient survival through HBXIP expression to emerge as a prognostic biomarker for this malignancy. The mechanistic aspects of the oncogenic roles of HBXIP in HCC necessitate further probing and scrutiny.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Drs. Guo and Zhu proposed the concept and designed the study, and Dr. Jiang contributed to the acquisition of data. All authors provided input to the manuscript. All authors read and approved the final manuscript.

Conceptualization: Ziyi Guo. Data curation: Ziyi Guo. Formal analysis: Ziyi Guo. Methodology: Lipeng Jiang. Software: Ziyi Guo. Supervision: Zhitu Zhu. Validation: Lipeng Jiang. Writing – original draft: Ziyi Guo, Zhitu Zhu. Writing – review & editing: Ziyi Guo, Lipeng Jiang, Zhitu Zhu.

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