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Original Article

Low expression of LACTB promotes tumor progression and predicts poor prognosis in hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is a major life-threatening malignancy worldwide. HCC has an unfavorable prognosis, mainly due to late diagnosis, early metastasis, and post-surgical recurrence. Recent studies have demonstrated that beta-lactamases (LACTB) plays a pivotal role in the pathogenesis and progression of several malignant tumors, but its expression and functional role in HCC has not been reported. In this study, we explored the expression of LACTB using The Cancer Genome Atlas datasets and two independent tissues microarrays. We then analyzed the correlation between LACTB expression and clinical outcomes in HCC. We demonstrated that LACTB mRNA and protein levels were both down-regulated in HCC, and decreased LACTB expression was associated with TNM stage, histologic grade, and overall survival of patients. Additionally, through Gene Set Enrichment Analysis, we found that the genes negatively related to the survival of HCC patients were enriched in the low LACTB expression group. Furthermore, we confirmed that overexpression of LACTB inhibited HCC cell proliferation, invasion, and migration *in vitro*, as well as decreased tumor growth *in vivo*. Online prediction results suggested that the LACTB gene was markedly correlated with genes involved in the lipid metabolism pathway. In conclusion, these findings suggest that down-regulated LACTB could function as a novel biomarker for diagnosis and prognosis prediction, and LACTB could serve as a promising target in HCC therapy.

Keywords: Beta-lactamases (LACTB), hepatocellular carcinoma (HCC), biomarker, prognosis

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver, especially in China [1]. Although improvements have been made in surgery and other treatment options for HCC [2-5], the 5-year overall survival rate remains unfavorable, mainly due to metastasis and recurrence. Given the limitations of current HCC therapies, it is critical to define the molecular mechanisms of hepatocarcinogenesis and develop novel strategies for early diagnosis and prognosis prediction in HCC.

Metabolic dysregulation is essential for progression in cancers, including HCC [6]. Recent reports have shown that aberrant lipid metabolism plays an essential role in HCC [7]. Beta-lactamases (LACTBs), mainly located in mitochondrion [8], are ubiquitous in several human tissues, especially in skeletal, heart, muscle and liver [9]. As a mammalian active-site serine protein, LACTB is evolutionarily related to bacterial penicillin-binding and B-lactamase proteins [10]. The mitochondrial gene LACTB functions as a novel protease homologue involved in the regulation of metabolic circuitry and cellular metabolic processes [11]. In addition, LACTB is a regulator of intra-mitochondrial membrane organization and energy homeostasis [8]. Recent studies reveal that LACTB can serve as a tumor suppressor through regulating lipid metabolism in breast cancer [11]. Moreover, studies show that LACTB is frequently down-regulated in breast cancer [11, 12], colorectal cancer [13], and glioma [14]. Additionally, overexpression of LACTB can suppress tumor cell proliferation. These findings demonstrate that LACTB is a novel, epigenetically silenced tumor suppressor. However, the expression and the functional roles of LACTB in HCC remain largely unexplored.

Table 1. The relationship between LACTB expression and clinicopathological features of ZZU HCC cohort

	Clinicopathological features	No. of cases	LACTB expression		*P*
			Low (n = 181)	High (n = 160)	
Age (years)	≤50	126	65	61	0.673
	>50	215	116	99	
Gender	Male	265	141	124	0.929
	Female	76	40	36	
Liver cirrhosis	Yes	315	168	147	0.743
	No	26	13	13	
Tumor size	≤5 cm	172	78	94	0.004*
	>5 cm	169	103	66	
TNM stage	Stage I and II	263	120	143	0.000**
	Stage III and IV	78	61	17	
Portal vein thrombosis	Absent	269	132	137	0.000**
	Present	72	49	23	

Abbreviations: TNM = tumor-node-metastasis; LACTB = beta-lactamases, *P<0.05, **P<0.001.

In our previous study utilizing a limited tumor sample size, we found that LACTB expression was frequently dysregulated in several cancers, including HCC [12]. In the present study, we observed that LACTB mRNA and protein levels were down-regulated in a relatively large number of HCC tumor samples. Additionally, we confirmed that LACTB expression was significantly associated with TNM stage, histological grade, and overall survival in HCC by The Cancer Genome Atlas (TCGA) data analysis and tissue microarray (TMA) analysis. Furthermore, we confirmed that overexpression of LACTB could inhibit HCC cell proliferation, migration, and invasion *in vitro*, as well as decrease tumor growth *in vivo*. Online prediction was performed to assess the association between LACTB and key enzymes in the pathway of lipid metabolism. Taken together, our findings suggest that LACTB may contribute to tumor progression in HCC, implying that LACTB can be considered a potential prognostic biomarker and a novel therapeutic target in HCC.

Materials and methods

TCGA data set analysis

Data from 374 liver cancer samples and 50 non-tumor samples from The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/tcga/) database was adopted in this study for gene expression analysis and patients' survival analyses.

Patients

A total of 396 patients (341 with available follow-up data) who were diagnosed with HCC in the First Affiliated Hospital of Zhengzhou University (ZZU HCC cohort) from 2011 to 2015 were selected for this study. All enrolled patients met the inclusion following criteria: distinctive pathologic diagnosis of HCC. Clinicopathological data are presented in **Table 1**. The present study was approved by the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University, and written informed consent was obtained from each patient.

Tissue microarray construction

The TMA of the ZZU HCC cohort contained 396 HCC tissues and paired adjacent normal tissues, and was constructed using 1.5-mm diameter cores. Another TMA containing 84 HCC specimens and 75 paired non-tumor specimens (Outdo HCC cohort) was purchased from Outdo Biotech (Shanghai, China).

Immunohistochemical (IHC) staining

The IHC was performed as described previously [15]. The images of IHC staining were obtained and analyzed. LACTB was scored according to staining intensity from 1+ to 5+. A score 1+ to 3+ was defined as low LACTB expression, whereas a score 4+ to 5+ was defined as high LACTB expression.

Cell lines and cell culture

SMMC-7721 and Hep3B cells were obtained from the Academy of Sciences (Shanghai, China), cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA), and incubated in a CO_2 incubator (5% CO_2/95% air) at 37°C. Details concerning the cell lines are shown in Table S1.

Western blotting

The Western blotting procedure was performed as previously described [16]. Total protein isolation from HCC cells was performed with RIPA buffer, and the protein concentration was determined. Protein samples (20 μg) were separated using a 12% SDS-PAGE gel (Invitrogen, USA) and then transferred to a nitrocellulose membrane (Millipore, USA). The membranes were incubated with blocking buffer (Invitrogen, USA), followed by primary antibody. The membranes were washed three times with PBST, followed by a 1-hour incubation with an HRP-conjugated secondary antibody. Then the membranes were used to expose the photographic film. Primary antibodies used in this study are shown in Table S1.

Lentiviral transfection

The LACTB-expressing lenti-viruses (Lv-LACTB) used in our study were synthesized by Hanheng (Shanghai, China). Lv-LACTB or Lv-NC virus was transfected 24 hours after HCC cells were plated into 6-well plates. After 24 hours of virus exposure, the cells were screened with 1 μg/ml puromycin for 3 days to obtain transformants stably expressing LACTB. The transfected cells were collected for further research.

Cell proliferation and colony formation assay

Cell growth was detected by a CCK-8 Kit. Transfected and nontransfected HCC cells were seeded into a 96-well plate. At the established time point, 100 μL 2-(2-Methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfobenzene)-2H-tetrazolium monosodium salt (WST-8) (Beyotime, China) was added, the cells were incubated for 4 hours at 37°C, and then absorbance was read at 570 nm by spectrophotometer (Molecular Devices, USA).

A colony formation assay was utilized to determine the effect of down-regulated LACTB on HCC cell growth. We diluted and plated the transfected cells in 6-well plates at 2,000 cells per well. Following a 10 day incubation, colonies were fixed, stained, and counted.

Cell migration and invasion assay

A wound-healing assay was performed. Cells were seeded in a 6-well plate. A wound was scratched using 20 ul sterile plastic tips after the cells reached approximately 90% confluency, and dispensed their respective treatments subsequently. At the indicated time points, pictures were taken under the microscope.

A cell invasion assay was also performed. Briefly, 1×10^4 transfected cells were cultured on the upper chamber of a transwell insert in serum-free medium, with the bottom chamber containing DMEM with 10% FBS. After 24 hours, the invasive cells were stained for subsequent photographing and counting.

Tumor xenograft experiments

The SMMC-7721 cells were transfected with Lv-LACTB or Lv-NC. After selection with puromycin, (2×10^6) stable transfected SMMC-7721 cells were administered by a subcutaneous injection to male BALB/c nude mice (4 weeks old)purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China). Tumor growth was measured every week and photographed using the IVIS@ Lumina II system (Caliper Life Sciences, USA). Mice were sacrificed after 6 weeks to collect tumors. This research was approved by the Animal Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

Gene set enrichment analysis (GSEA)

GSEA was used to determine which gene sets were associated with LACTB expression in the TCGA dataset. The expression profiles of 374 samples from the TCGA dataset were grouped into two classes according to LACTB expression (low LACTB group and high LACTB group). GSEA v2.0 was used to determine whether the members of the gene sets from the MSigDB database v4.0 are randomly distributed at the top or bottom of the ranking.

Statistical analysis

GraphPad Prism software (version 7.0) or SPSS software (version 23.0) was adopted to analyze statistical data. Student's *t*-test was performed to detect the correlation of LACTB expression with clinicopathological characteristics. Univariate and multivariate Cox proportional hazard regression models was performed to analyze independent prognostic factors. Kaplan-Meier survival analysis was performed to detect the significance of available survival data from TCGA and TMA. A $P<0.05$ was considered statistically significant.

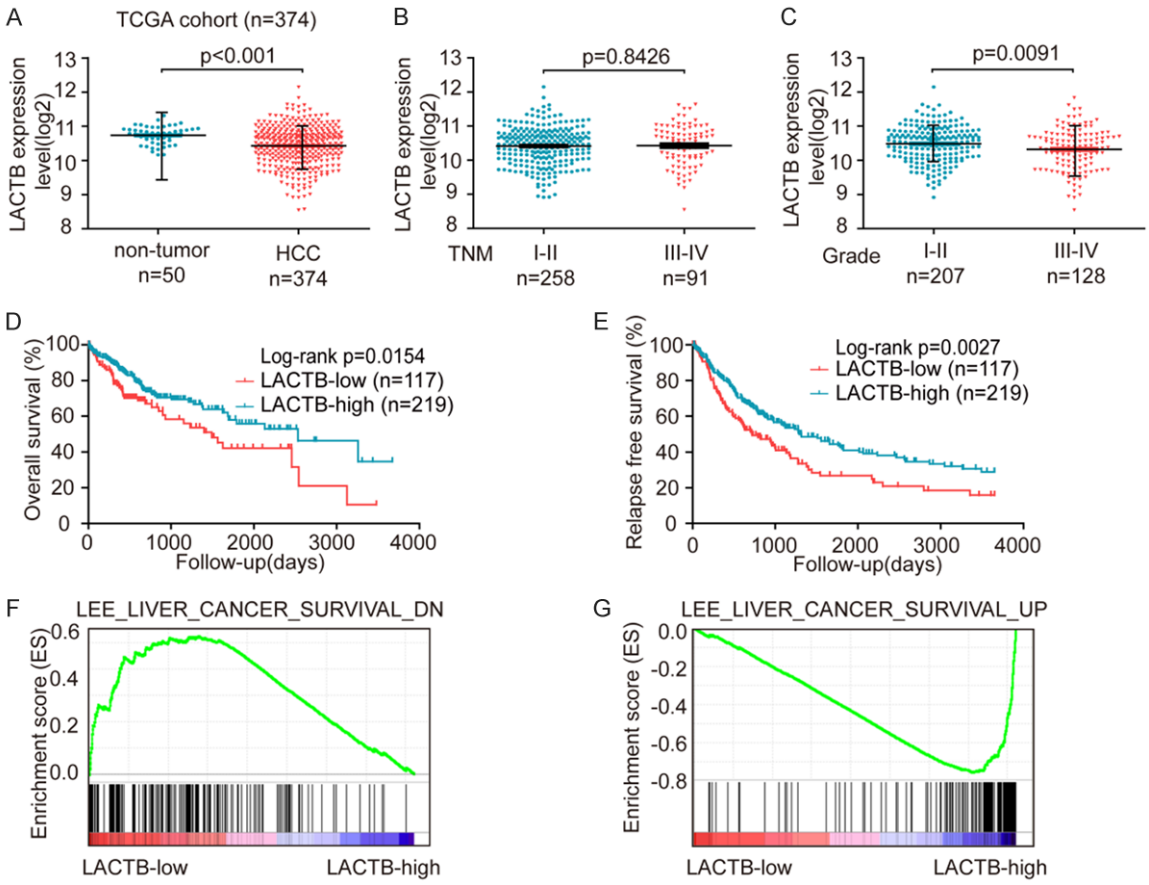

Figure 1. Low expression of LACTB is correlated with prognosis of HCC patients in the TCGA dataset. A. LACTB mRNA was down-regulated in HCC tissues in a TCGA data analysis. B, C. The expression of LACTB was much lower in high histological grade, whereas there was no obvious difference in TNM staging. D, E. Kaplan-Meier analysis of LACTB expression levels and prognosis of HCC patients. The results showed that the OS and RFS of HCC patients with low LACTB expression were significantly worse than those with high LACTB expression. F, G. A GSEA analysis showed that the genes negatively related to the survival of HCC patients were enriched in the low LACTB expression group, whereas the genes positively correlated with survival were enriched in the high LACTB expression group. OS: Overall survival; RFS: Relapse free survival.

Results

Low expression of LACTB is correlated with the prognosis of HCC patients in the TCGA dataset

To detect the clinical significance of LACTB expression in HCC, we analyzed TCGA datasets. Results showed that LACTB mRNA was down-regulated in HCC tissues compared to normal tissues (**Figure 1A**). Furthermore, we analyzed the correlation between LACTB expression and TNM stage and pathological grade in HCC patients using the TCGA database. We found that LACTB expression was much lower in patents with high histological grade, whereas there was no obvious difference in patients from different TNM stages (**Figure 1B** and **1C**). Furthermore, Kaplan-Meier analysis showed that the overall survival (OS) and disease-free survival (DFS) of HCC patients with low LACTB expression were significantly worse compared to those with high LACTB expression (**Figure 1D** and **1E**). Through GSEA analysis, we found that the genes negatively related to the survival of HCC patients were enriched in the low LACTB expression group, while the genes positively correlated with the survival were enriched in the high LACTB expression group (**Figure 1F** and **1G**). These findings suggested that LACTB mRNA was down-regulated and contributed to the unfavorable prognosis of HCC patients.

LACTB is down-regulated in HCC tissues and associated with poor prognosis

We then examined the protein expression of LACTB in HCC clinical samples from the Outdo HCC cohort. According to staining intensity in

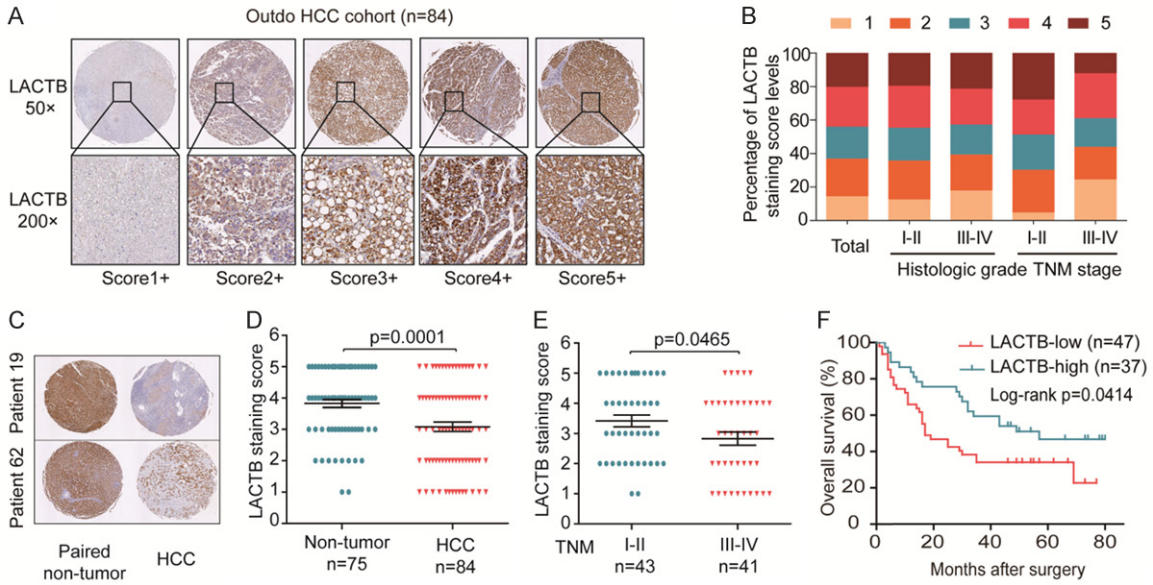

Figure 2. LACTB is down-regulated in HCC tissues and associated with poor prognosis in the Outdo HCC cohort. A. LACTB staining patterns were categorized into 5 groups according to staining intensity. B. The score distribution in patients with different histological grade and TNM stage was presented. C, D. Representative LACTB staining and histological scoring in HCC tissues and paired non-tumor samples. LACTB expression was low in HCC tissues compared with the non-tumor tissues. E. LACTB expression was low in HCC patients with advanced TNM stage (III-IV). F. Kaplan-Meier survival analysis comparing LACTB expression groups (red, high LACTB expression; green, low LACTB expression). HCC patients with low LACTB expression had a markedly shorter OS than those with high LACTB expression.

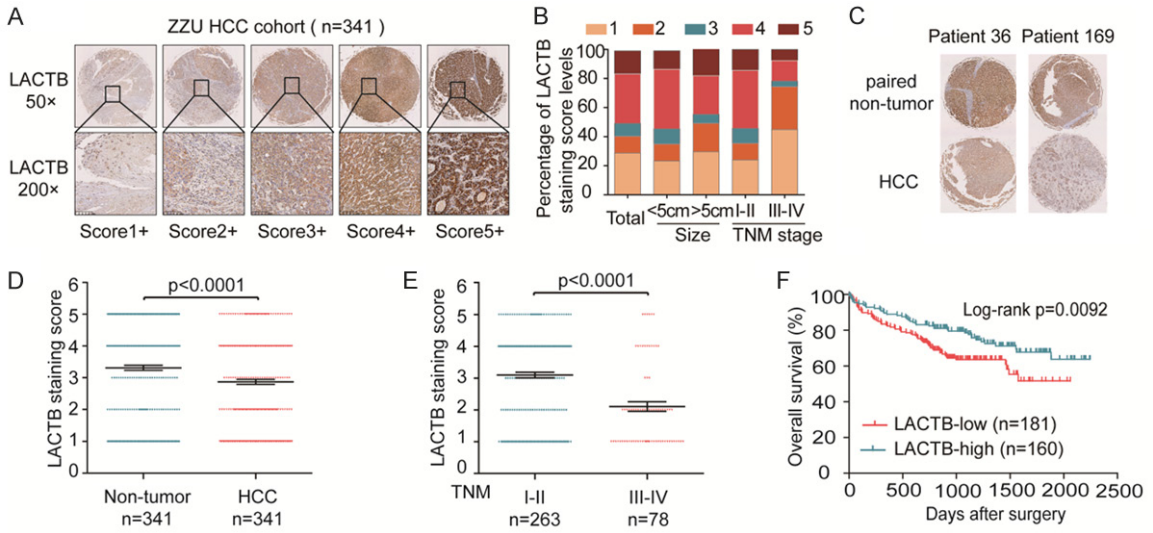

Figure 3. LACTB is down-regulated in HCC tissues and associated with poor prognosis in the ZZU HCC cohort. A. LACTB staining patterns were categorized into 5 groups according to staining intensity. B. The score distribution in patients with different tumor size and TNM stage was presented. C, D. Representative LACTB staining and histological scorings in HCC tissues compared to non-tumor samples. LACTB expression was low in HCC tissues compared with the non-tumor tissues. E. LACTB expression was notably low in HCC patients with advanced TNM stage. F. Kaplan-Meier survival analysis between expression of LACTB (red, high LACTB expression; green, low LACTB expression). HCC patients with low LACTB expression had markedly shorter OS than those with high LACTB expression.

the TMA, we categorized LACTB staining patterns into 5 groups (**Figure 2A**). **Figure 2B** presents the typical graphs of score distribution in patients. There is a significant difference

Table 2. Univariate and multivariate analyses of overall survival of the ZZU HCC cohort

	Clinicopathological features	Univariate analyses			Multivariate analyses		
		HR	95% (CI)	*P* value	HR	95% (CI)	*P* value
Age (years)	≤Median	1.000	0.809-1.195	0.863			
	>Median	0.983					
Gender	Male	1.000	0.745-1.871	0.479			
	Female	1.181					
Liver cirrhosis	Yes	1.000	0.812-2.825	0.192			
	No	1.515					
Tumor size	≤5 cm	1.000	0.390-0.835	0.004*	1.000	0.637-1.707	0.869
	>5 cm	0.571			1.562		
TNM stage	Stage I and II	1.000	0.204-0.442	0.000**	1.000	0.103-0.664	0.000**
	Stage III and IV	0.300			0.261		
Portal vein thrombosis	Absence	1.000	0.358-0.816	0.003*	1.000	0.702-1.857	0.594
	Presence	0.541			1.141		
LACTB expression	Low	1.000	2.908-7.134	0.000**	1.000	2.446-6.199	0.000**
	High	4.555			3.894		

Abbreviations: TNM = tumor-node-metastasis; LACTB = beta-lactamases, *P<0.05, **P<0.001.

between TNM stage I-II and TNM stage III-IV, whereas there is no difference between histological grades. Consistent with mRNA expression, LACTB protein expression was lower in the HCC tissues than in the matched adjacent non-tumor tissues (**Figure 2C** and **2D**). Interestingly, LACTB protein expression was much lower in later TNM stages (stage III and IV) (**Figure 2E**). Kaplan-Meier analysis demonstrated that low LACTB expression was significantly associated with poor OS in HCC (**Figure 2F**). To further evaluate LACTB protein expression in HCC, we assessed the ZZU HCC cohort (**Figure 3A**). **Figure 3B** presents the typical graphs of LACTB score distribution in patients, and there is a significant difference in tumor size and TNM stage. Consistent with the Outdo HCC cohort, there was a notable difference in LACTB expression between HCC tissues and non-tumor tissues, based on LACTB staining score (**Figure 3C**), and we found that LACTB expression was obviously decreased in HCC tissues (**Figure 3D**). Additionally, we found that LACTB expression was markedly decreased in HCC patients with TNM III-IV stage versus I-II stage (**Figure 3E**). Kaplan-Meier analysis also showed that low LACTB expression patients had a significantly poorer OS when compared to high LACTB expression patients (**Figure 3F**). Taken together, these results validated that LACTB was significantly down-regulated and associated with poor clinical outcomes in HCC.

Low expression of LACTB is an independent adverse prognostic factor in HCC patients

Subsequently, a Student's *t*-test demonstrated that there were significant associations between LACTB levels in the ZZU HCC cohort and tumor size, TNM stage, and portal vein thrombosis, whereas there was no relationship between LACTB levels in the cohort and age, gender, or liver cirrhosis (**Table 1**). In this cohort, univariate and multivariate analysis suggested that decreased LACTB expression and TNM stage were independent prognostic factors for predicting poor survival of HCC patients (**Table 2**). Similar results were observed for the Outdo HCC cohort, as shown in Tables S2 and S3. These results demonstrate that decreased LACTB expression serves as a poor prognosis biomarker for HCC patients.

Overexpression of LACTB inhibits HCC cell proliferation, migration, and invasion in vitro

To explore the biological function of LACTB, we performed *in vitro* experiments using HCC cells. By western blot, we found that LACTB was markedly increased in the Lv-LACTB group. (**Figure 4A**). The effect of LACTB up-regulation on HCC cell proliferation was affirmed by CCK-8 assay and EDU assay (**Figure 4B** and **4C**). The colony formation assay demonstrated that overexpression of LACTB significantly decreased the colony-formation ability of both SMMC-

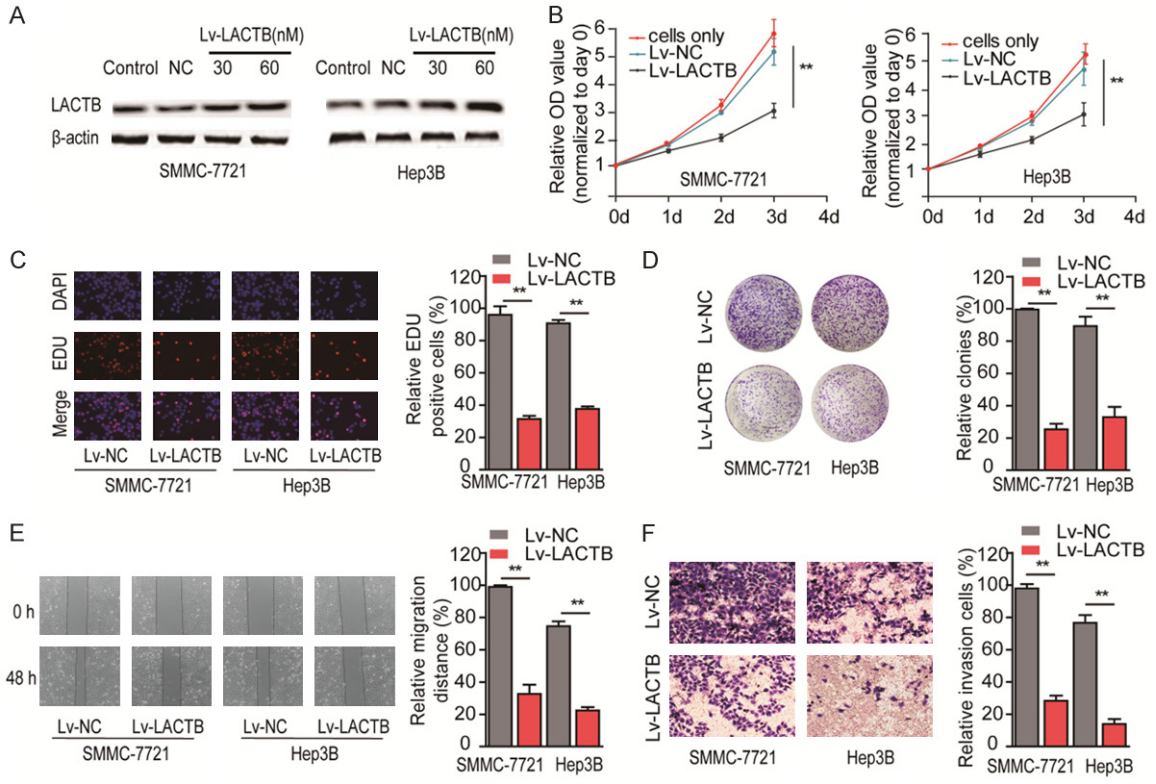

Figure 4. Overexpression of LACTB inhibits HCC cell proliferation, migration, and invasion *in vitro*. (A) Western blot confirmed that LACTB expression was significantly increased after transfection with LACTB-expressing Lenti-viruses (Lv-LACTB) in SMMC-7721 and Hep3B cells. The CCK8 assay (B) and EdU assay (C) showed that treatment with Lv-LACTB could inhibit cell proliferation ability of SMMC-7721 and Hep3B cells. (D) The number of colonies was significantly decreased for cells transfected with Lv-LACTB compared to respective controls. (E) Wound-healing assay showed that overexpression of LACTB could cause a remarkable suppression of cell migration in SMMC-7721 and Hep3B cells. (F) The invasiveness of SMMC-7721 and Hep3B cells treated with Lv-LACTB was significantly suppressed according to a cell invasion assay. *P<0.05, **P<0.001.

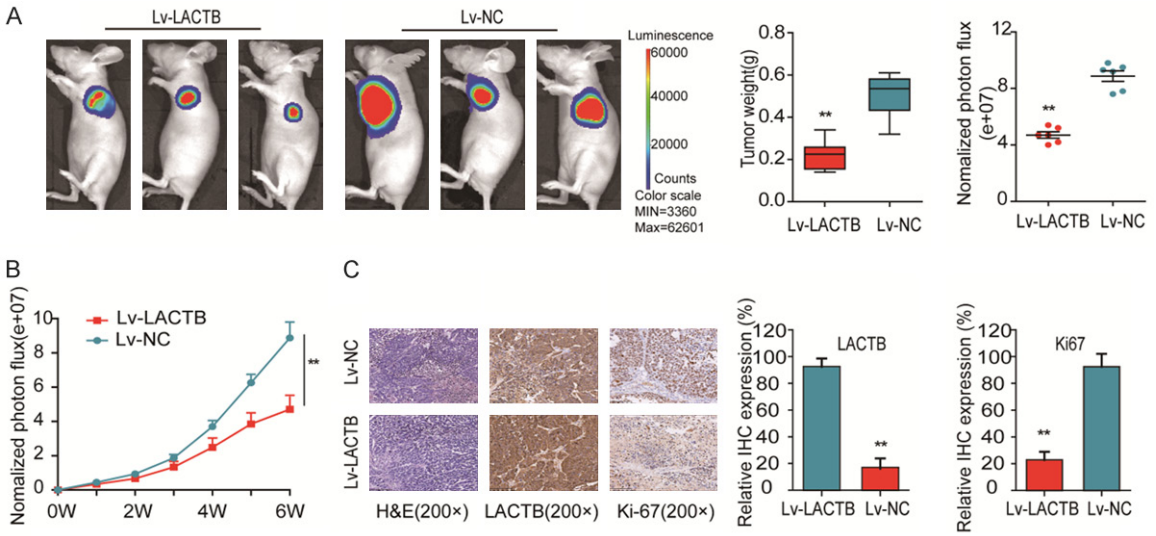

Figure 5. Overexpression of LACTB suppresses tumor growth *in vivo*. A. Tumor weight and tumor volume in the Lv-LACTB group were markedly smaller than those of Lv-NC group. B. A live imaging system was utilized to exam the luciferase signal. The luciferase activity of the Lv-LACTB tumors was lower than that of the Lv-NC group. C. Sections of xenograft tumors stained with immunohistochemical staining for LACTB and Ki-67, as well as HE staining. The expression of LACTB was much higher in the Lv-LACTB group than the Lv-NC group by IHC staining, while the Ki-67 of Lv-NC group was increased compared with the Lv-LACTB group. *P<0.05, **P<0.001.

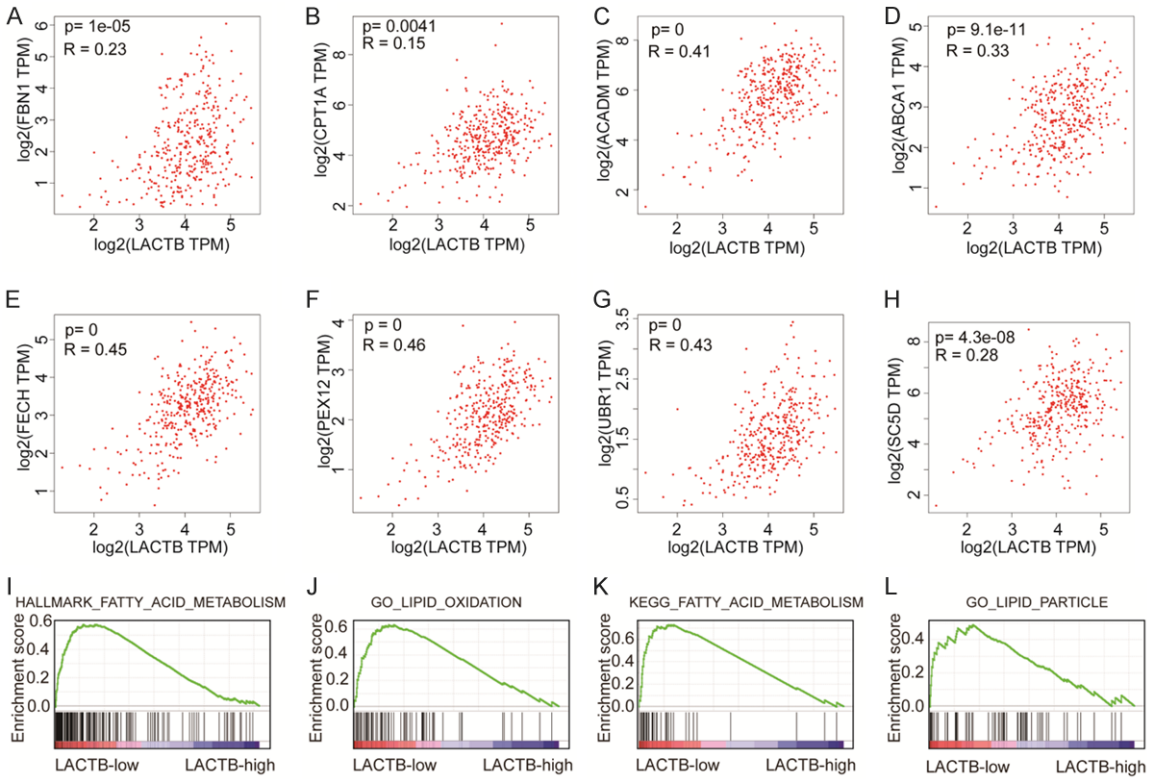

Figure 6. LACTB has potential roles in lipid metabolism. A-H. Key enzymes in the pathway of lipid metabolism (FBN1, CPT1A, ACADM, ABCA1, FECH, PEX12, UBR1, and SC5D) in HCC were significantly related with LACTB by GEPIA analysis. I-L. Online prediction results showed that the expressed LACTB was markedly correlated with genes involved in the lipid metabolism pathway.

7721 and Hep3B cells (**Figure 4D**). In parallel, the decreased migration and invasion abilities of SMMC-7721 and Hep3B cells treated with Lv-LACTB were confirmed by wound healing and transwell invasion assays, respectively (**Figure 4E** and **4F**). Based on these findings, the up-regulation of LACTB inhibited HCC cell proliferation, invasion, and migration, suggesting that LACTB plays vital roles in HCC progression.

Overexpression of LACTB suppresses tumor growth in vivo

We further examined the effects of changes in LACTB expression *in vivo*, and results showed that tumor size was notably decreased in the Lv-LACTB group compared to the Lv-NC group (**Figure 5A** and **5B**). Utilizing IHC staining, we also observed that LACTB expression was much higher in the Lv-LACTB group than the Lv-NC group, whereas Ki-67 expression was increased in the Lv-NC group compared with the Lv-LACTB group (**Figure 5C**). Collectively, overexpression of LACTB could suppress tumorigenesis *in vivo*, indicating that LACTB might be a promising target in HCC therapy.

LACTB has potential roles in lipid metabolism

To detect the functional roles of LACTB in lipid metabolism, we performed an online prediction to assess the association between LACTB and key enzymes of the lipid metabolism pathway. As shown in **Figure 6A-H**, LACTB was significantly related with FBN1, CPT1A, ACADM, ABCA1, FECH, PEX12, UBR1, and SC5D, which are reported components of the lipid metabolism pathway. Furthermore, online prediction results showed that the LACTB gene was markedly correlated with genes involved in the lipid metabolism pathway (**Figure 6I-L**). Taken together, these results suggest that LACTB may play essential roles in lipid metabolism.

Discussion

Dysregulated LACTB is significantly associated with obesity and atherosclerosis, demonstrat-

ing that LACTB might be involved in fat metabolism disorders [17]. Decreased LACTB expression is observed in different tumors, including gliomas [14], breast cancer [11, 12], and colorectal cancer [13]. To our knowledge, the present study is the first to thoroughly evaluate LACTB expression levels in HCC. Consistent with previous studies, we found that LACTB had lower expression at both the mRNA and protein level. Through TCGA dataset analysis, we found that the expression level of LACTB was decreased in HCC tissues compared with non-tumor tissues, which was confirmed by TMA analysis of two independent HCC cohorts. These results suggested that LACTB expression was frequently decreased in HCC tissues.

Furthermore, clinicopathological analysis suggested that decreased LACTB expression was markedly correlated with TNM stage, histologic grade, and OS of HCC patients. Moreover, through GSEA analysis we observed that genes negatively related to the survival of HCC patients were enriched in the low LACTB expression group. In agreement with our results, low expression of LACTB is associated with poor OS in colorectal cancer [13], breast cancer [12], and glioma [14]. Collectively, these results demonstrated that down-regulation of LACTB was involved in tumor progression, and that LACTB could serve as a promising therapeutic target in HCC.

In addition, univariate and multivariate analysis showed that down-regulated LACTB and late TNM stage were independent factors for poor prognosis in HCC, which suggested that low LACTB expression was associated with aggressive behavior and unfavorable prognosis in HCC. Similar results were observed in glioma and breast cancer. Li *et al.* confirms that LACTB expression is notably decreased in glioma patients and that decreased LACTB is correlated with a poor prognosis [14]. Zhang *et al.* reported that down-regulated LACTB expression is an independent prognostic factor for predicting poor survival in breast cancer [12]. These findings demonstrated that down-regulated LACTB could serve as a prognostic biomarker in HCC.

Importantly, we observed that overexpression of LACTB could suppress cell growth, migration, and invasion *in vitro,* as well as tumor growth *in vivo*. Overexpression of LACTB can also inhibit the proliferation, invasion, and angiogenesis of glioma cells [14]. Zeng *et al.* reports that overexpression of LACTB can suppress colorectal cancer cell proliferation, migration, and invasion *in vitro,* and tumor growth and metastasis *in vivo* [13]. These results demonstrated that LACTB may play a pivotal role in tumor development.

Metabolic reprogramming is a hallmark and driver of cancer [18-21]. Increasing evidence shows that abnormal fatty acid metabolism contributes to progression in cancers [22-24], including HCC [25-28]. For example, Senni *et al.* reports that β-catenin-activated HCC is driven by intensively oxidized fatty acids instead of glycolysis [29]. Guri *et al.* reports that elevated mTORC2 promotes HCC via the formation of lipids essential for growth and energy production [30]. A previous study confirms that LACTB can modulate lipid metabolism to inhibit the proliferation of breast cancer cells [11]. Consistent with results in breast cancer, our Pearson correlation analysis showed that LACTB expression level was closely related to the hub genes of lipid metabolism, while GSEA showed a significant enrichment in the lipid metabolism pathway. These results indicated that LACTB may play essential roles in HCC carcinogenesis and progression through the regulation of lipid metabolism processes, but our findings need confirmation through additional studies.

Conclusion

Our findings demonstrated for the first time that LACTB levels in HCC are down-regulated and associated with poor outcomes. LACTB may function as a tumor suppressor in HCC and could serve as a novel prototype therapeutic agent and a potential biomarker for HCC patients.

Acknowledgements

The study was approved by the human ethic committee of the First Affiliated Hospital of Zhengzhou University. All patients provided written informed consent and the project was in accordance with the Helsinki Declaration of 1975. Their clinical information is kept in the databases of the First Affiliated Hospital of Zhengzhou University and utilized for research. All the *in vivo* experiments were approved by the institutional animal care and use commit-

tee of the First Affiliated Hospital of Zhengzhou University.

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Disclosure of conflict of interest

None.

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Table S1. Information on antibodies used in this study

Antibody	WB	IHC	Specificity	Company
β-actin	1:5000	/	Mouse monoclonal	Proteintech Group, China
LACTB	1:1000	1:100	Rabbit Polyclonal	Proteintech Group, China
Ki-67	/	1:500	Rabbit Polyclonal	Proteintech Group, China

Table S2. The relationship between LACTB expression and clinicopathological features of Outdo HCC cohort

	Clinicopathological features	No. of cases	LACTB expression		*P*
			Low (n = 47)	High (n = 37)	
Age (years)	≤Median	28	16	12	0.876
	>Median	56	31	25	
Gender	Male	76	42	34	0.695
	Female	8	5	3	
Size	≤5 cm	38	20	18	0.577
	>5 cm	46	27	19	
TNM stage	Stage I and II	43	19	24	0.026*
	Stage III and IV	41	28	13	
Histological grade	Grade 1-2	56	31	25	0.876
	Grade 3-4	28	16	12	

Abbreviations: TNM = tumor-node-metastasis; LACTB = Lactamase Beta, *P<0.05.

Table S3. Univariate and multivariate analyses of overall survival of Outdo HCC cohort

	Clinicopathological features	Univariate analyses			Multivariate analyses		
		HR	95% (CI)	*P* value	HR	95% (CI)	*P* value
Age (years)	≤Median	1.000	0.963-1.009	0.215			
	>Median	0.985					
Gender	Male	1.000	0.155-1.605	0.244			
	Female	0.499					
Tumor size	≤5 cm	1.000	1.022-3.217	0.042*	1.000	0.514-2.025	0.954
	>5 cm	1.814			1.021		
TNM stage	Stage I and II	1.000	1.543-4.919	0.001*	1.000	1.231-4.979	0.011*
	Stage III and IV	2.755			2.475		
Histological grade	Grade 1-2	1.000	0.684-2.161	0.505			
	Grade 3-4	1.216					
LACTB expression	Low	1.000	0.522-0.816	0.000**	1.000	0.551-0.852	0.001*
	High	0.653			0.685		

Abbreviations: TNM = tumor-node-metastasis; HR = hazard ratio; CI = confidential interval; LACTB = Lactamase Beta, *P<0.05, **P<0.001.