


# Circular RNA Hsa\_circ\_0062403 Acts as a Potential Prognostic Biomarker for Early Recurrence of Hepatocellular Carcinoma

Xi Yu, Yuying Shan, Caide Lu, Shuqi Mao 

Department of Hepatopancreatobiliary Surgery, Ningbo Medical Center Lihuli Hospital, Ningbo Zhejiang 315040 People's Republic of China

Correspondence: Shuqi Mao; Caide Lu, Department of Hepatopancreatobiliary Surgery, Ningbo Medical Center Lihuli Hospital, Ningbo, Zhejiang, 315040, People's Republic of China, Email [mmaoshuqi@163.com](mailto:mmmaoshuqi@163.com); [lucaide@nbu.edu.cn](mailto:lucaide@nbu.edu.cn)

**Purpose:** Circular RNAs (circRNAs) have been shown to have critical regulatory roles in hepatocellular carcinoma (HCC). This study aims to investigate the predictive value of circLZTR1 for early recurrence of HCC.

**Patients and Methods:** In this study, circLZTR1 (a circRNA derived from the LZTR1, hsa\_circ\_0062403) was identified and validated in the HCC cohort. Univariate analyses and multivariate Cox regression were conducted to analyze the functional role of hsa\_circ\_0062403 in the postoperative early recurrence of HCC patients. Receiver operating characteristic curve (ROC) analysis was performed to assess the predictive performance of hsa\_circ\_0062403 for early recurrence.

**Results:** 91 patients with HCC after surgery were included. circLZTR1 was downregulated in early recurrence HCC patients and predicted poor recurrence-free survival ( $P=0.029$ ). In addition, a significant downregulation of circLZTR1 was observed in HCC from patients with MVI or PVTT. Multivariate Cox regression analysis indicated that lower circLZTR1 ( $>0.25$  vs  $<0.1$ ; HR = 0.142, 95% CI: 0.042–0.482,  $P = 0.002$ ), higher AFP ( $>400$  vs  $<20$ ; HR = 7.880, 95% CI: 2.499–24.844,  $P < 0.001$ ), higher INR (HR = 4.203, 95% CI: 1.133–15.588,  $P = 0.032$ ), multiple tumors (HR = 3.795, 95% CI: 1.541–9.346,  $P = 0.004$ ), PVTT positive (HR = 3.438, 95% CI: 1.050–11.258,  $P = 0.041$ ), and larger tumor diameter (HR = 1.306, 95% CI: 1.101–1.550,  $P = 0.002$ ) served as independent risk factor for early recurrence of HCC. ROC analysis indicated that the Cox model demonstrated higher sensitivity and specificity than the clinical model and single circLZTR1 in prediction for early recurrence of HCC patients (AUC = 0.767, 95% CI: 0.666–0.868,  $P < 0.001$ ).

**Conclusion:** The downregulation of circLZTR1 is closely associated with the acquisition of invasive phenotypes, vascular invasion, and subsequent early recurrence of HCC patients, suggesting that circLZTR1 may serve as a novel prognostic biomarker for predicting early recurrence in patients with HCC.

**Keywords:** circular RNA, early recurrence, hepatocellular carcinoma, prognostic biomarker

## Introduction

Primary liver cancer is the sixth most frequently diagnosed cancer and the third leading cause of cancer death worldwide, according to global cancer statistics 2022.<sup>1</sup> Primary liver cancer mainly includes 75%–85% hepatocellular carcinoma (HCC) and 10%–15% intrahepatic cholangiocarcinoma (ICC). And 21%–55% of liver cancer cases are mainly related to chronic hepatitis B and C virus infection.<sup>2,3</sup> In contrast to the declining proportion of virus-related HCC, the incidence of non-viral HCC, particularly that driven by metabolic dysfunction-associated steatotic liver disease (MASLD), is increasing rapidly. MASLD affects 38% of the global population, and it is estimated that the global prevalence of MASLD will reach 56% within the next decade.<sup>4</sup> However, HCC is frequently diagnosed at an advanced stage, thus precluding surgical resection and conferring a poor prognosis.<sup>5</sup> Recurrence and metastasis remain the leading causes of poor prognosis and long-term mortality in patients with resectable HCC. Despite remarkable advances in the treatment of hepatocellular carcinoma (HCC) in recent years—including interventional therapy, local ablation, chemotherapy, targeted therapy, and immunotherapy—the prognosis of patients with HCC remains dismal, with a 5-year overall survival rate of less than 18%.<sup>6</sup>

Circular RNAs (circRNAs) are a unique class of non-coding RNAs (ncRNAs) defined by their covalently closed loop structure, a hallmark feature that distinguishes them from linear ncRNA species. CircRNAs exert pivotal regulatory roles in transcriptional and post-transcriptional gene expression via diverse mechanisms, including acting as miRNA sponges, encoding functional polypeptides, serving as protein scaffolds, and forming stable RNA-protein complexes to modulate downstream biological processes.<sup>7</sup> It is well documented that dysregulated circRNAs are critically involved in the tumorigenesis and malignant progression of diverse human cancers, including HCC,<sup>8–10</sup> lung cancer,<sup>11,12</sup> gastric cancer,<sup>13</sup> breast cancer,<sup>14,15</sup> colorectal cancer,<sup>16</sup> hematological malignancies,<sup>17</sup> and neuroblastoma,<sup>18</sup> and are indispensable modulators of the establishment and maintenance of tumor drug-resistant phenotype.<sup>19</sup> Given that dysregulated circRNAs are stably expressed and readily detectable in tissues, peripheral blood, and body fluids, circRNAs hold great promise to serve as novel biomarkers and therapeutic intervention targets for the diagnosis and treatment of malignant tumors.<sup>20</sup> Tang et al<sup>21</sup> indicated that circRNA\_0009792 enhances the stemness of HCC cells via the PSIP1/c-Myc signaling pathway, and ultimately drives HCC progression and the development of lenvatinib resistance. In another study, researchers found that higher circMRCK $\alpha$  levels presented significantly reduced overall survival and greater cumulative recurrence. circMRCK $\alpha$  promotes tumor glycolysis and progression via USP22 binding and HIF-1 $\alpha$  upregulation.<sup>22</sup> These findings highlight that circRNAs, acting as new biomarkers, exert critical regulatory roles in the recurrence and metastasis of HCC; however, further research is still needed.

In this study, we identified a novel circRNA (hsa\_circ\_0062403) in HCC, which was named circLZTR1, which is encoded by the Leucine zipper-like post-translational regulator 1 (*LZTR1*) gene. In our present study, we revealed that the knockdown of *LZTR1* can promote HCC cell proliferation and metastasis, and might be used as an effective predictive marker for HCC recurrence. Mechanistically, *LZTR1* downregulation increased RAS protein expression, leading to MAPK pathway activation and enhanced epithelial-mesenchymal transition. Although a substantial number of circRNAs have been documented to participate in the tumorigenesis and malignant progression of HCC, few studies have specifically focused on early postoperative recurrence. Furthermore, the biological function and clinical value of circLZTR1 in HCC early recurrence remain largely unreported. Therefore, in this study, we aim to investigate the predictive value of circLZTR1 for early recurrence of HCC, and to clarify its intrinsic correlation with the malignant biological phenotype of HCC.

## Materials and Methods

### Patients and Tissues

91 HCC patients who underwent surgical resection were included in this study. The inclusion criteria are as follows: (1) All patients were pathologically diagnosed with HCC; (2) Not receiving radiotherapy, immunotherapy, or chemotherapy before surgery. The exclusion criteria were as follows: (1) history of other cancers; and (2) incomplete clinical or follow-up data. Early recurrence of HCC was uniformly defined as tumor recurrence and metastasis occurring within 1 year after radical hepatectomy for HCC. This study process was approved by the Ethics Committee of the Ningbo Medical Center Lihuili Hospital (Ethical approval number KY2022PJ020). All tissue samples were collected during liver cancer surgical resection, stored at low temperature ( $-80^{\circ}\text{C}$ ) with RNA Preservation Kit (RNAhold, TransGen Biotech, Beijing, China).

### RNA Extraction, Reverse Transcription, and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from HCC tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration and purity of total RNA were verified using the NANO Quant infinite M200PRO spectrophotometer (TECAN, Männedorf, Switzerland). Complementary DNA (cDNA) was synthesized via reverse transcription using the AMV Reverse Transcription System (Invitrogen, Carlsbad, CA, USA) with 2  $\mu\text{g}$  of qualified total RNA as input template.

qRT-PCR was performed with 2 $\times$ PCR Mix (QIAGEN, Hilden, Germany) on the ABI ViiA 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. For circular RNA expression analysis, back-spliced junction-specific primers for circLZTR1 were designed. The primer sequences for hsa\_circ\_0062403 were 5'-TACATGCCAAGGGCCCTAAA-3' (forward) and 5'-CGGCTGGAGAAACCACATTT-3'

(reverse). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the endogenous normalizer, with primer sequences of 5'-AGCCACATCGCTCAGACAC-3' (forward) and 5'-GCCCAATACGACCAAATCC-3' (reverse). All primers were synthesized by Shanghai Jierui Bioengineering Co., Ltd. (Shanghai, China). For the low abundance of circRNA, a 12-cycle pre-amplification was performed prior to formal qRT-PCR amplification. qRT-PCR was carried out in triplicate for each sample, and the specificity of amplification was confirmed via melt curve analysis. The relative expression level of circLZTR1 was calculated using the  $2^{-\Delta\Delta C_t}$  method.<sup>23</sup>

## Statistical Analysis

Statistical analyses were performed using SPSS 25.0 (IBM Corporation, 2020, USA). Numerical data were expressed as means and standard deviations (SDs) for normally distributed data, while categorical data were expressed as numbers (%). We used the chi-square test and *t* test to compare the clinicopathological characteristics and circLZTR1 levels between the early recurrence group and the non-early recurrence group (Control). Patients were divided into different risk groups (Low-; Moderate-; High-) with the cut-off points of circLZTR1 automatically calculated by X-tile software using an exhaustive method and Log rank test (Yale University, CT, USA). Subsequently, Survival curves were calculated to compare the recurrence-free survival (RFS) rates in three different risk groups using the Kaplan-Meier method and the Log rank test. A multivariate Cox proportional hazards regression model was performed to identify predictors of early recurrence. Variables showing significant differences in univariate *t* or  $\chi^2$ -tests (*P*-value < 0.05) were included in the multivariate Cox regression model for identification of independent prognostic factors. Receiver operating characteristic curve (ROC) analysis was performed to assess the predictive performance of circLZTR1 for early recurrence. Statistical significance was defined as *P*-value < 0.05.

## Results

### Baseline Characteristics of Enrolled HCC Patients

The baseline characteristics are shown in Table 1. In this cohort, 75/91 (82%) patients were male, and 16/91 (18%) patients were female. 27/91 (29.7%) patients were diagnosed with early recurrence within 1 year after radical resection. The average age of the early recurrence group was 59.5 years (59.5±13.8), 61.4 years (61.4±12.3) in the control.

**Table 1** Baseline Characteristics of the HCC Patients

Characteristic	Control (N=64)	Early Recurrence (N=27)	<i>t</i> / $\chi^2$	<i>P</i>
Gender (male/female)	53/11	22/5	0.023	0.879
Age	61.4±12.3	59.5±13.8	0.645	0.521
HBV (negative/positive)	14/50	1/26	4.555	0.033
Anti-HBV (no/yes)	32/32	13/14	0.026	0.872
AFP grade (<20/20-400/>400)	38/19/7	9/10/8	6.840	0.033
PT, seconds (≤12.5/>12.5)	48/16	20/7	0.009	0.926
TT, seconds (≤16.6/>16.6)	46/18	17/10	0.708	0.400
APTT (≤36.5/>36.5)	61/3	26/1	0.044	0.834
DB, umol/l (≤8/>8)	58/6	19/8	5.984	0.014
ALT, U/L (≤50/>50)	56/8	23/4	0.089	0.766
ALP, U/L (≤125/>125)	59/5	22/5	2.225	0.136
GGT, U/L (≤60/>60)	46/18	16/11	1.392	0.238

(Continued)

**Table 1** (Continued).

Characteristic	Control (N=64)	Early Recurrence (N=27)	$t/\chi^2$	P
INR ( $\leq 1 / > 1$ )	20/44	3/24	4.078	0.043
ALBI ( $< -2.6 / -2.6 - 1.39$ )	46/18	13/14	4.689	0.30
AST, U/L ( $\leq 40 / > 40$ )	52/12	18/9	2.275	0.131
Pathological differentiation (poor/moderate/well)	20/40/3	15/10/2	5.374	0.068
MVI (negative/positive)	44/20	12/15	4.740	0.029
Liver cirrhosis (negative/positive)	15/49	5/22	0.268	0.605
Tumor diameter, cm	3.99 $\pm$ 2.09	5.49 $\pm$ 3.55	2.504	0.014
Number of tumors (single/multiple)	54/10	14/13	10.635	0.001
PVTT (no/yes)	62/2	22/5	6.337	0.012
AJCC T stage (I / II / III / IV)	44/14/5/1	10/5/5/7	18.123	<0.001
CircLZTR1 ( $< 0.1 / 0.1 - 0.25 / > 0.25$ )	8/14/42	9/8/10	7.600	0.022

**Abbreviations:** HBV, hepatitis B virus; AFP, alpha-fetoprotein; PT, prothrombin time; TT, thrombin time; APTT, activated partial thromboplastin time; DB, direct bilirubin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT,  $\gamma$ -glutamyl transpeptidase; INR, international normalized ratio; ALBI, albumin- bilirubin; AST, aspartate aminotransferase; MVI, microvascular invasion; PVTT, portal vein tumor thrombosis; AJCC, American Joint Committee on Cancer; LZTR1, Leucine zipper-like post-translational regulator 1.

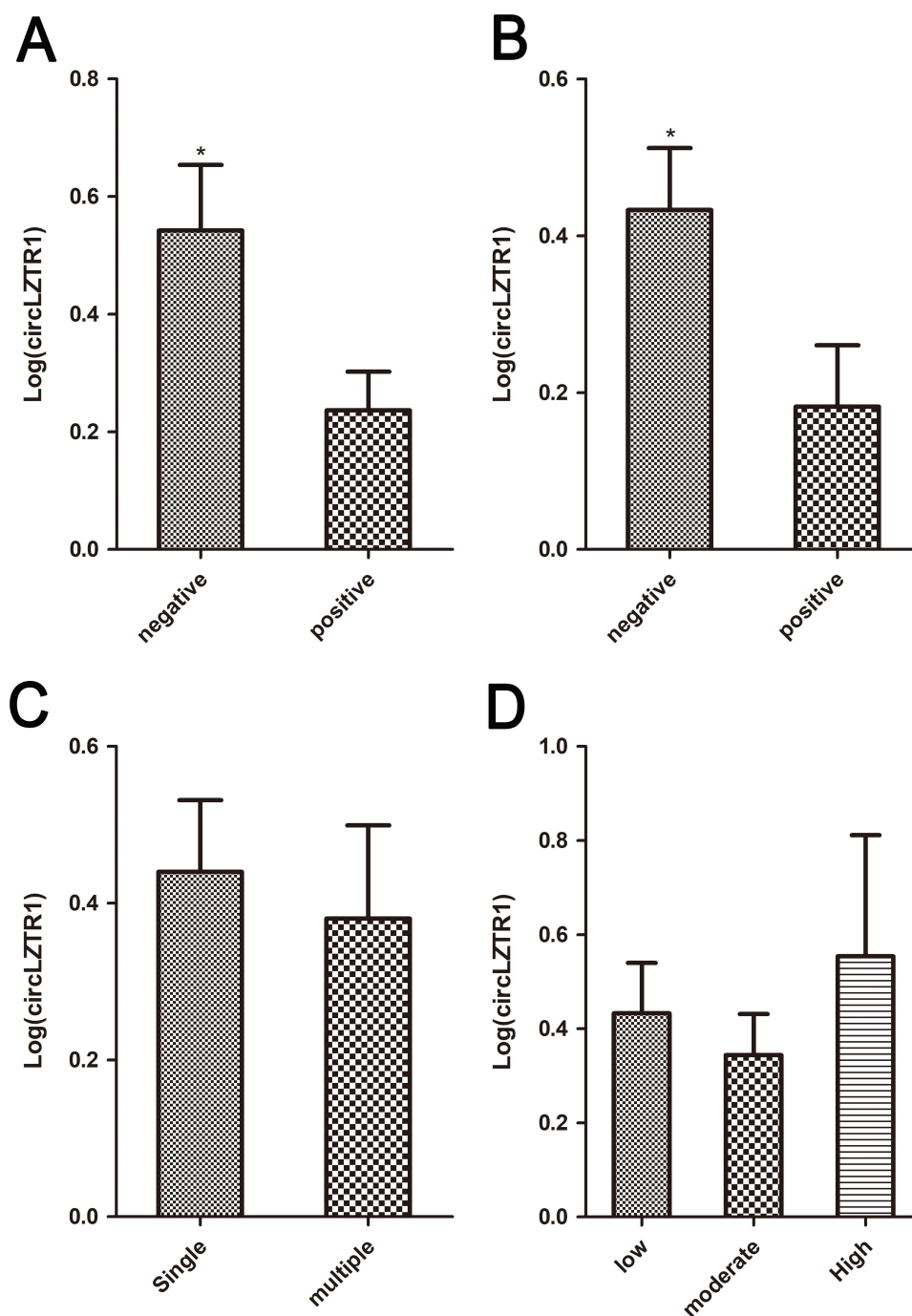
Regarding tumor profiles, 35/91 (38.5%) were MVI positive, 7/91 (7.7%) were PVTT positive, 18 (19.8%) had tumors in the AJCC T3 and T4 stages, and 35/91 (38.5%) had poor pathological differentiation. The chi-square and  $t$  test found that hepatitis B virus (HBV) infection, alpha-fetoprotein (AFP) grade, direct bilirubin (DB), international normalized ratio (INR), microvascular invasion (MVI), tumor diameter, number of tumors, and portal vein tumor thrombosis (PVTT) were significant difference between early recurrence and control group ( $P < 0.05$ ). In addition, the lower circLZTR1 expression level was also observed in MVI and PVTT positive patients, as shown in [Figure 1](#) ( $P < 0.05$ ). Post-hoc power analysis showed a power of 0.82 for independent  $t$ -test and 0.998 for Log rank test by circLZTR1 expression level.

## Risk Stratification and Prognostic Assessment for circLZTR1

The optimal cut-off points of circLZTR1 expression level were auto-calculated by the X-tile software and divided HCC patients into the high risk group ( $< 0.10$ ), moderate risk group ( $0.10 - 0.25$ ), and low risk group ( $> 0.25$ ). The chi-square test indicated that there was a significant difference between the early recurrence and control group ( $\chi^2 = 7.60$ ;  $P = 0.022$ ). Survival curves were calculated to compare the RFS rates in three different risk groups using the Kaplan-Meier method, and the results showed a significant discriminatory ability for recurrence risks ( $P < 0.05$ , [Figure 2](#)).

## Prognostic Factors for Early Recurrence in Multivariate Cox Regression Analysis

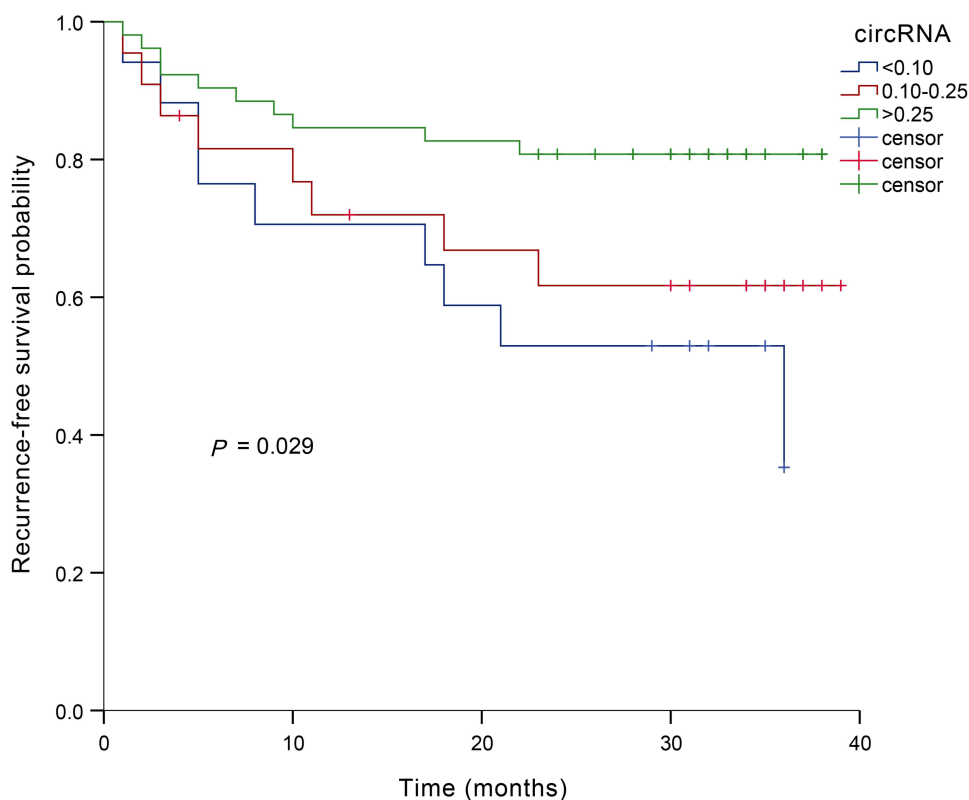
Based on the results of univariable analysis in [Table 1](#), variables with statistical significance ( $P < 0.05$ ) were entered into the multivariable Cox proportional hazards regression model, including HBV infection, AFP grade, DB, INR, MVI, tumor diameter, number of tumors, PVTT and circLZTR1. Multivariable Cox regression analysis showed that AFP grade ( $> 400$  vs  $< 20$ : HR = 7.880, 95% CI: 2.499–24.844,  $P < 0.001$ ), INR ( $> 1$  vs  $\leq 1$ : HR = 4.203, 95% CI: 1.133–15.588,  $P = 0.032$ ), number of tumors (HR = 3.795, 95% CI: 1.541–9.346,  $P = 0.004$ ), PVTT (HR = 3.438, 95% CI: 1.050–11.258,  $P = 0.041$ ), tumor diameter (HR = 1.306, 95% CI: 1.101–1.550,  $P = 0.002$ ), and circLZTR1 ( $> 0.25$  vs  $< 0.10$ : HR = 0.142, 95% CI: 0.042–0.482,  $P = 0.002$ ) were independent influence factors for HCC early recurrence ([Table 2](#)). The results indicated that a lower circLZTR level was an independent risk factor for HCC early recurrence.



**Figure 1** The association between circLZTR1 and HCC malignant phenotypes. (A) Association with MVI. \*P < 0.05 (B) Association with PVTT. \*P < 0.05 (C) Association with tumor number. (D) Association with AFP levels.

## Predictive Performance of circLZTR1 for Early Recurrence by ROC Analysis

We established three prognostic models for predicting HCC early recurrence based on multivariable Cox regression analysis results: ①Clinical Model, a clinicopathological feature-based model incorporating AFP grade, INR, tumor number, PVTT, and tumor diameter; (2) CircLZTR1 Model, a univariable model with circLZTR1 expression as the only predictor; (3) Cox Model, the full model integrating all the above clinicopathological variables and circLZTR1. ROC curve analysis was conducted to assess the predictive performance of each model and AJCC T stage, with the area under the curve (AUC) calculated to quantify the predictive accuracy. The results revealed that the circLZTR1 alone model



**Figure 2** Recurrence-free survival curve of circLZTR1 in three different risk groups.

exhibited a favorable predictive value for early HCC recurrence (AUC = 0.661, 95% CI: 0.534–0.787, P = 0.016), with an AUC comparable to that of the Clinical Model (AUC = 0.655, 95% CI: 0.534–0.776, P = 0.020). More importantly, the combined Cox Model that incorporated circLZTR1 into the clinicopathological features (AUC = 0.767, 95% CI: 0.666–0.868, P < 0.001) yielded a significantly higher AUC than the Clinical Model and AJCC T stage (AUC = 0.707, 95% CI: 0.579–0.835, P = 0.002), indicating a remarkably enhanced predictive efficacy for early recurrence in HCC patients. The results were shown in Table 3 and Figure 3.

**Table 2** Multivariate COX Analyse for Early Recurrence of HCC Patients

Characteristic	B	SE	Wald	Varianc	P	HR (95% CI)
HBV (positive vs negative)	1.338	1.082	1.531	1.000	0.216	3.813 (0.458–31.755)
AFP, ng/mL			12.415	2.000	0.002	
20-400 vs <20	1.035	0.536	3.725	1.000	0.054	2.814 (0.984–8.047)
>400 vs <20	2.064	0.586	12.413	1.000	<0.001	7.880 (2.499–24.844)
DB, umol/l (>8 vs ≤8)	0.694	0.564	1.513	1.000	0.219	2.001 (0.662–6.046)
INR (>1 vs ≤1)	1.436	0.669	4.609	1.000	0.032	4.203 (1.133–15.588)
MVI (positive vs negative)	−0.010	0.510	0.000	1.000	0.984	0.990 (0.364–2.691)
Number of tumors (multiple vs single)	1.334	0.460	8.410	1.000	0.004	3.795 (1.541–9.346)
PVTT (positive vs negative)	1.235	0.605	4.162	1.000	0.041	3.438 (1.050–11.258)

(Continued)

**Table 2** (Continued).

Characteristic	B	SE	Wald	Varianc	P	HR (95% CI)
Tumor diameter, cm	0.267	0.087	9.358	1.000	0.002	1.306 (1.101–1.550)
CircLZTR1			11.190	2.000	0.004	
0.1–0.25 vs <0.1	–0.537	0.592	0.823	1.000	0.364	0.585 (0.183–1.864)
>0.25 vs <0.1	–1.953	0.625	9.777	1.000	0.002	0.142 (0.042–0.482)

**Abbreviations:** HBV, hepatitis B virus; AFP, alpha-fetoprotein; DB, direct bilirubin; INR, international normalized ratio; MVI, microvascular invasion; PVTT, portal vein tumor thrombosis; LZTR1, Leucine zipper-like post-translational regulator 1.

**Table 3** Comparison of ROC Curve with and Without circLZTR1

Characteristic	Sensitivity	Specificity	AUC (95% CI)	P
Cox model	0.781	0.743	0.767 (0.666–0.868)	<0.001
Clinical model	0.500	0.852	0.655 (0.534–0.776)	0.020
CircLZTR1	0.656	0.630	0.661 (0.534–0.787)	0.016
AJCC T stage	0.906	0.444	0.707 (0.579–0.835)	0.002

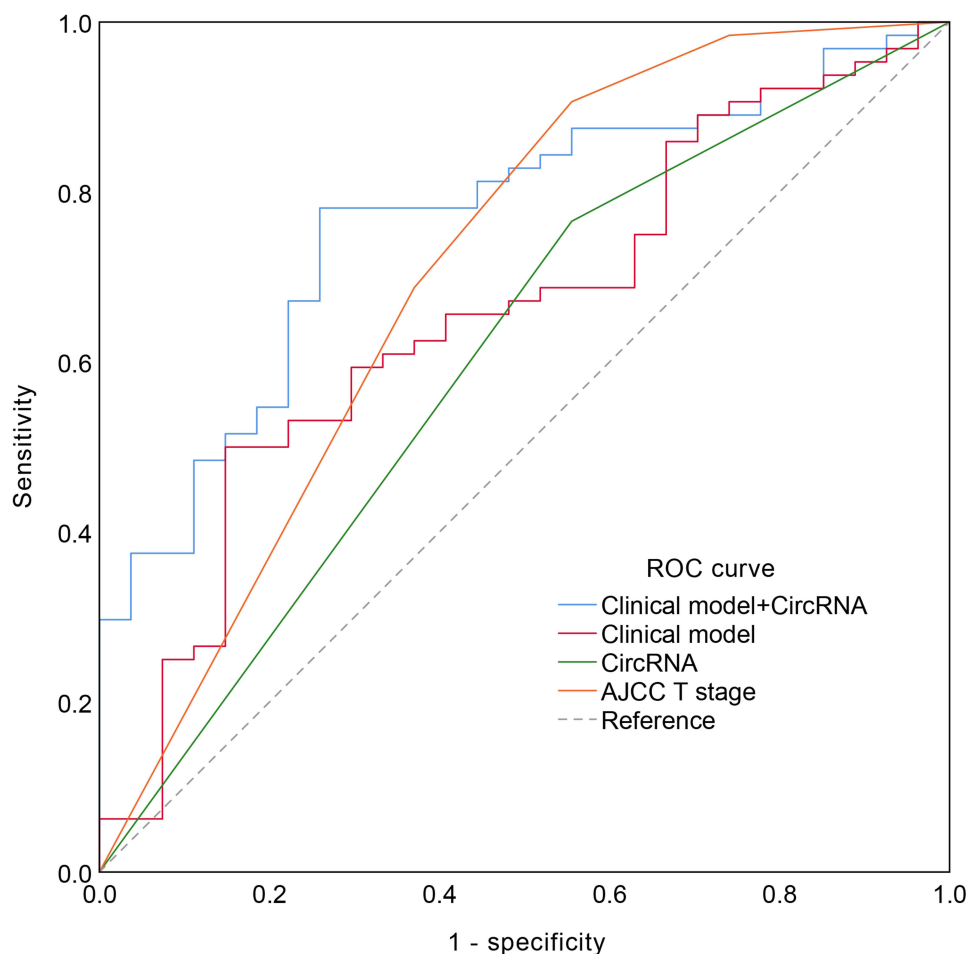
**Abbreviation:** LZTR1, Leucine zipper-like post-translational regulator 1.

## Discussion

CircRNAs are characterized by a single-stranded, covalently closed circular structure, which lacks 5' caps and 3' poly(A) tails as a class of non-coding RNAs. This unique feature renders them resistant to ribonuclease degradation, with a significantly longer half-life than linear mRNAs. CircRNAs can regulate the transcriptional and post-transcriptional expression of their host genes, modulate pre-mRNA alternative splicing, serve as competing endogenous RNAs (ceRNAs) to sponge microRNAs (miRNAs), bind and sequester RNA-binding proteins (RBPs), and act as scaffolds to orchestrate protein-protein interactions.<sup>24</sup> Beyond these well-established roles, a growing body of evidence has revealed that a subset of circRNAs can be translated into functional peptides or proteins via cap-independent translation pathways.<sup>25</sup> In this study, we found that lower circLZTR1 expression level was closely associated with the MVI and PVTT, and may serve as a novel, promising prognostic biomarker for predicting early recurrence in patients with HCC. In our present study, the knockdown of *LZTR1* can promote HCC cell proliferation and metastasis, and was associated with early recurrence of HCC. Mechanistically, *LZTR1* downregulation increased RAS protein expression, leading to MAPK pathway activation and enhanced epithelial-mesenchymal transition.<sup>26</sup> Consequently, we put forward the hypothesis that circLZTR1 may exert its recurrence-promoting and pro-metastatic effects in HCC predominantly through regulating the expression of its cognate host gene *LZTR1*.

Kurahashi et al<sup>27</sup> first reported that *LZTR1* was encoded by a gene mapping to chromosome 22q11.2, and identified via sequence homology with leucine zipper-containing proteins. Subsequent studies identified *LZTR1* as a member of the BTB–Kelch superfamily. Functionally, *LZTR1* acts as the substrate-specific adaptor component of the Cullin 3 (CUL3) ubiquitin ligase complex, which drives the ubiquitination and subsequent proteasomal degradation of canonical RAS proteins as well as the non-canonical RAS-family member RIT1, and consequently restrains the activation of the RAS/MAPK signaling pathway.<sup>28–30</sup> However, the RAS/MAPK signaling pathway plays a fundamental role in the malignant progression and short survival of human HCC.<sup>31</sup> Yıldız et al<sup>32</sup> identified that the loss of the *LZTR1* gene can reduce the vimentin expression and motility, and then exhibited reduced transwell migration and invasion in *LZTR1* knockout (KO) Hep3B HCC Cells.

In this study, we first found that circLZTR1 (hsa\_circ\_0062403), a circular RNA derived from the back-splicing of its host gene *LZTR1*, acts as a promising prognostic biomarker for predicting early recurrence of postoperative HCC



**Figure 3** Receiver operating characteristic curve of circLZTR1 in prediction for HCC early recurrence.

patients. Our previous study<sup>26</sup> demonstrated that *LZTR1* downregulation markedly activates the oncogenic RAS/MAPK cascade and induces epithelial-mesenchymal transition (EMT), both of which are core events driving HCC malignant progression, metastasis, and early recurrence, as Zhao et al indicated.<sup>33</sup> These findings support the clinical value of circLZTR1 and imply that it may participate in HCC progression via regulating *LZTR1* and its downstream signaling. Notably, this potential mechanistic linkage is merely a scientific hypothesis deduced from previously published evidence, rather than a conclusion verified by functional experiments in the present study. In addition, we also observed a significant downregulation of circLZTR1, which was associated with MVI or PVTT, and may serve as a critical mediator of HCC malignant phenotypes. As Yıldız et al<sup>32</sup> reported, the loss of *LZTR1* protein could remodel EMT and mesenchymal plasticity (EMP). Notably, profound intratumoral heterogeneity and dynamic phenotypic plasticity are core drivers of tumor progression and metastatic dissemination in HCC.<sup>34</sup> Mounting evidence confirmed that EMT enhanced the invasiveness of circulating tumor cells (CTCs) and played a pivotal role in promoting HCC metastasis and MVI progression.<sup>35–37</sup> Therefore, we hypothesize that circLZTR1 may promote the malignant progression and metastatic dissemination of HCC by regulating the EMT and EMP process. However, the specific functional mechanism of circLZTR1 in HCC, including whether it exerts biological effects by directly regulating its host gene, acting as a miRNA sponge, or interacting with functional proteins, has not yet been elucidated. Further in-depth studies are still needed to clarify its underlying regulatory network.

Accumulating evidence indicates that circRNAs can serve as important biomarkers for predicting poor prognosis in HCC patients. Jang et al<sup>38</sup> discovered that high *hsa\_circ\_0003570* expression was an independent prognostic factor for progression-free survival (PFS, HR=0.633). In another study, high *hsa\_circ\_0004018* and *hsa\_circ\_0003570* were

inversely independent risk factors for PFS in patients with HBV-HCC.<sup>39</sup> A study on cholangiocarcinoma (CCA) complicated with biliary obstruction reported that a CCA-specific circRNA panel consisting of hsa\_circ\_0000367, hsa\_circ\_0021647, and hsa\_circ\_0000288, which can be detected in both bile and serum exosomes, has been identified as a promising biomarker for postoperative early recurrence monitoring of CCA.<sup>40</sup> Our study first found that circLZTR1 can act as an important biomarker for early recurrence (1-year) of HCC (ACU = 0.767), this result providing a novel approach for preoperative risk stratification and postoperative surveillance of HCC.

Certainly, there are some limitations of this study as follows: First, this study was a single-center retrospective study with a relatively small sample size of 91 HCC patients, which may lead to inherent selection bias and limited statistical power, and needs to be further validated in larger multi-center cohorts. Second, we only proposed the potential regulatory mechanism of circLZTR1 in HCC based on our previous functional research on its host gene *LZTR1*, while systematic in vitro and in vivo functional experiments and mechanistic validation at cellular and animal levels have not been performed. Third, circLZTR1 expression was not validated in non-invasive body fluid samples, including serum, plasma, and urine, which limits the exploration of its clinical translational potential as a liquid biopsy biomarker.

In conclusion, the downregulation of circLZTR1 is closely associated with the acquisition of invasive phenotypes, vascular invasion, and subsequent early recurrence of HCC patients after surgery, suggesting that circLZTR1 may serve as a novel, potential prognostic biomarker for predicting early recurrence in patients with HCC. Given the limitations of the present study, large-scale cohorts, external validation and mechanistic research are warranted to confirm our observations.

## Conclusion

In conclusion, the downregulation of circLZTR1 is closely associated with the acquisition of invasive phenotypes, vascular invasion, and subsequent early recurrence of HCC patients after surgery, suggesting that circLZTR1 may serve as a novel, promising prognostic biomarker for predicting early recurrence in patients with HCC.

## Data Sharing Statement

The datasets used and/or analyzed during the current study available from the corresponding author (Shuqi Mao) on reasonable request.

## Ethics Approval

This study process was approved by the Ethics Committee of the Ningbo Medical Center Lihuili Hospital (Ethical approval number KY2022PJ020).

## Informed Consent

As this was a retrospective observational study, the requirement for informed consent was waived by the Ethics Committee of Lihuili Hospital. All patient data were anonymized and de-identified prior to analysis to protect patient privacy and confidentiality.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This study was supported by Ningbo Top Medical and Health Research Program (2024020818), The Ningbo Major Research and Development Plan Project (2023Z160/2024Z179), Science Foundation of Lihuili Hospital (2022KYPYXM-L2), Ningbo Natural Science Foundation (2022J262/2024J393), and Project of NINGBO Leading Medical & Health Discipline (2026-A10).

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74(3):229–263. doi:10.3322/caac.21834
2. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health.* 2020;8(2):e180–e190. doi:10.1016/s2214-109x(19)30488-7
3. Runggay H, Ferlay J, de Martel C, et al. Global, regional and national burden of primary liver cancer by subtype. *Eur J Cancer.* 2022;161:108–118. doi:10.1016/j.ejca.2021.11.023
4. Estes C, Anstee QM, Arias-Loste MT, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030. *J Hepatol.* 2018;69(4):896–904. doi:10.1016/j.jhep.2018.05.036
5. Runggay H, Arnold M, Ferlay J, et al. Global burden of primary liver cancer in 2020 and predictions to 2040. *J Hepatol.* 2022;77(6):1598–1606. doi:10.1016/j.jhep.2022.08.021
6. Liu Z, Wang Y, Wang Y, Zhang Y. Exosomal circRNAs in hepatocellular carcinoma: implications for the development and therapeutic resistance of hepatocellular carcinoma (Review). *Int J Oncol.* 2026;68(2):26. doi:10.3892/ijo.2025.5839
7. Liu CX, Chen LL. Circular RNAs: characterization, cellular roles, and applications. *Cell.* 2022;185(12):2016–2034. doi:10.1016/j.cell.2022.04.021
8. Jazbec G, Režen T. Circular RNA in hepatocellular carcinoma: emerging therapeutic strategies. *Front Immunol.* 2025;16:1722576. doi:10.3389/fimmu.2025.1722576
9. Huang Y, Xu L, Yang L, et al. Targeting circGDI2 disrupt HNRNPC-mediated mPORCN stabilization and enhance LGK-974 anti-tumor therapy in hepatocellular carcinoma. *Mol Cancer.* 2026;25(1):110. doi:10.1186/s12943-026-02638-1
10. Xue S, Wei D, Zhao Y, et al. CircRSU1 activates the hnRNPA1/HIF-1 $\alpha$ /CD24 signaling axis, promoting stemness features of hepatocellular carcinoma. *Adv Sci.* 2026;13(22):e22424. doi:10.1002/advs.202522424
11. Li J, Zhang Q, Jiang D, et al. CircRNAs in lung cancer- role and clinical application. *Cancer Lett.* 2022;544:215810. doi:10.1016/j.canlet.2022.215810
12. Peng W, Chen K, Hu Y, et al. circTNIK promotes carbon nanotubes-induced lung carcinogenesis via GRP78-mediated endoplasmic reticulum stress and suppression of Type I interferon signaling. *ACS Nano.* 2026;20(6):4808–4827. doi:10.1021/acsnano.5c16536
13. Yang R, Xu B. The role of circular RNA in the occurrence and progression of gastric cancer: emerging perspectives on its use as diagnostic and therapeutic biomarkers and therapeutic targets. *Int J Biol Macromol.* 2026;336:149442. doi:10.1016/j.ijbiomac.2025.149442
14. Liu G, Liu Q, Jia L, et al. Exosomal circRNAs: key modulators in breast cancer progression. *Cell Death Discov.* 2025;11(1):196. doi:10.1038/s41420-025-02494-w
15. Xu J, Xu Q, Cao T, et al. The interaction of CircESR1 and HNRNPAB regulates cell cycle transition of breast cancer cell. *Int J Biol Sci.* 2026;22(3):1520–1541. doi:10.7150/ijbs.126014
16. Behera R, Khandelwal A, Devi V, et al. Circular RNAs in colorectal cancer: emerging insights and future perspectives. *Biochem Pharmacol.* 2026;243(Pt 1):117495. doi:10.1016/j.bcp.2025.117495
17. Deng F, Zhang C, Lu T, et al. Roles of circRNAs in hematological malignancies. *Biomark Res.* 2022;10(1):50. doi:10.1186/s40364-022-00392-2
18. Wu K, Tan J, Yang C. Recent advances and application value of circRNA in neuroblastoma. *Front Oncol.* 2023;13:1180300. doi:10.3389/fonc.2023.1180300
19. Liu W, Niu J, Huo Y, et al. Role of circular RNAs in cancer therapy resistance. *Mol Cancer.* 2025;24(1):55. doi:10.1186/s12943-025-02254-5
20. Verduci L, Tarcitano E, Strano S, Yarden Y, Blandino G. CircRNAs: role in human diseases and potential use as biomarkers. *Cell Death Dis.* 2021;12(5):468. doi:10.1038/s41419-021-03743-3
21. Tang Y, Yuan F, Cao M, et al. CircRNA-mTOR promotes hepatocellular carcinoma progression and lenvatinib resistance through the PSIP1/c-Myc axis. *Adv Sci.* 2025;12(20):e2410591. doi:10.1002/advs.202410591
22. Yu S, Su S, Wang P, et al. Tumor-associated macrophage-induced circMRCK $\alpha$  encodes a peptide to promote glycolysis and progression in hepatocellular carcinoma. *Cancer Lett.* 2024;591:216872. doi:10.1016/j.canlet.2024.216872
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>(-Delta Delta C(T))</sup> method. *Methods.* 2001;25(4):402–408. doi:10.1006/meth.2001.1262
24. Kristensen LS, Andersen MS, Stagsted LVW, et al. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet.* 2019;20(11):675–691. doi:10.1038/s41576-019-0158-7
25. Tajik Z, Ghafouri-Fard S. Circular RNAs: key regulators of paclitaxel sensitivity and resistance in cancer. *Eur J Med Res.* 2025;30(1):758. doi:10.1186/s40001-025-03045-w
26. Yu X, Mao S, Shan Y, et al. LZTR1 expression as a novel predictive biomarker for early recurrence of hepatocellular carcinoma. *Eur J Med Res.* 2025;30(1):500. doi:10.1186/s40001-025-02791-1
27. Kurahashi H, Akagi K, Inazawa J, et al. Isolation and characterization of a novel gene deleted in DiGeorge syndrome. *Hum Mol Genet.* 1995;4(4):541–549. doi:10.1093/hmg/4.4.541
28. Bigenzahn JW, Collu GM, Karting F, et al. LZTR1 is a regulator of RAS ubiquitination and signaling. *Science.* 2018;362(6419):1171–1177. doi:10.1126/science.aap8210
29. Steklov M, Pandolfi S, Baietti MF, et al. Mutations in LZTR1 drive human disease by dysregulating RAS ubiquitination. *Science.* 2018;362(6419):1177–1182. doi:10.1126/science.aap7607
30. Castel P, Cheng A, Cuevas-Navarro A, et al. RIT1 oncoproteins escape LZTR1-mediated proteolysis. *Science.* 2019;363(6432):1226–1230. doi:10.1126/science.aav1444
31. Delire B, Stärkel P. The Ras/MAPK pathway and hepatocarcinoma: pathogenesis and therapeutic implications. *Eur J Clin Invest.* 2015;45(6):609–623. doi:10.1111/eci.12441

32. Yıldız G, Karabulut S, Uzun U, et al. LZTR1 loss reduces vimentin expression and motility in Hep3B hepatocellular carcinoma cells. *Int J Mol Sci.* 2026;27(4):1866. doi:10.3390/ijms27041866
33. Zhao X, Wang Y, Xia H, et al. Roles and molecular mechanisms of biomarkers in hepatocellular carcinoma with microvascular invasion: a review. *J Clin Transl Hepatol.* 2023;11(5):1170–1183. doi:10.14218/jcth.2022.00013s
34. Chan LK, Tsui YM, Ho DW, Ng IO. Cellular heterogeneity and plasticity in liver cancer. *Semin Cancer Biol.* 2022;82:134–149. doi:10.1016/j.semcancer.2021.02.015
35. Orrapin S, Udomruk S, Lapisatepun W, et al. Clinical implication of circulating tumor cells expressing epithelial mesenchymal transition (EMT) and cancer stem cell (CSC) markers and their perspective in HCC: a systematic review. *Cancers.* 2022;14(14):3373. doi:10.3390/cancers14143373
36. Williams ED, Gao D, Redfern A, Thompson EW. Controversies around epithelial-mesenchymal plasticity in cancer metastasis. *Nat Rev Cancer.* 2019;19(12):716–732. doi:10.1038/s41568-019-0213-x
37. Li CF, Chen JY, Ho YH, et al. Snail-induced claudin-11 prompts collective migration for tumour progression. *Nat Cell Biol Feb.* 2019;21(2):251–262. doi:10.1038/s41556-018-0268-z
38. Jang SY, Kim G, Tak WY, et al. Circular noncoding RNA hsa\_circ\_0003570 as a prognostic biomarker for hepatocellular carcinoma. *Genes.* 2022;13(8):1484. doi:10.3390/genes13081484
39. Kang MK, Kim G, Park JG, et al. Tissue circular RNA\_0004018 and 0003570 as novel prognostic biomarkers for hepatitis B-related hepatocellular carcinoma. *Genes.* 2023;14(10):1963. doi:10.3390/genes14101963
40. Wen N, Peng D, Xiong X, et al. Cholangiocarcinoma combined with biliary obstruction: an exosomal circRNA signature for diagnosis and early recurrence monitoring. *Signal Transduct Target Ther.* 2024;9(1):107. doi:10.1038/s41392-024-01814-3

International Journal of General Medicine

Publish your work in this journal

The International Journal of General Medicine is an international, peer-reviewed open-access journal that focuses on general and internal medicine, pathogenesis, epidemiology, diagnosis, monitoring and treatment protocols. The journal is characterized by the rapid reporting of reviews, original research and clinical studies across all disease areas. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/international-journal-of-general-medicine-journal>

**Dovepress**  
Taylor & Francis Group