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Original Article

LIN28 expression and prognostic value in hepatocellular carcinoma patients who meet the Milan criteria and undergo hepatectomy

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Abstract

Stem cell marker *LIN28*, related closely with SOX2 and OCT4, has been studied as a biomarker for the maintainance of pluripotent cells in several malignancies. Our previous study showed that SOX2 and OCT4 were negative predictors for hepatocellular carcinoma (HCC). However, the predictive value of *LIN28* in HCC outcome is still undetermined. We hypothesized that *LIN28* may also play a role as a biomarker for HCC. To test this hypothesis, we examined the expression of *LIN28* in 129 radically resected HCC tissues using reverse transcription-polymerase chain reaction and analyzed the association of *LIN28* expression with clinicopathologic features and prognosis. Our study showed that *LIN28* was expressed at a higher frequency in tumor tissues than in non-HCC tissues (45.0% vs. 21.7%, $P = 0.020$). Moreover, *LIN28* expression was significantly increased in cases with large tumor size ($P = 0.010$). Univariate analysis did not reveal a significant correlation between *LIN28* expression and overall survival or recurrence-free survival. For HCC patients who met the Milan criteria, stratified analysis revealed shorter overall survival ($P = 0.007$) and recurrence-free survival ($P < 0.001$) in those with detectable *LIN28* expression compared to those with no detectable *LIN28* expression. Furthermore, multivariate analysis revealed that *LIN28* was a negative independent predictor for both overall survival (hazard ratio= 7.093, $P = 0.017$) and recurrence-free survival (hazard ratio=5.518, $P = 0.004$) in patients who met the Milan criteria. Taken together, our results suggest that *LIN28* identifies low-risk and high-risk subsets of HCC patients meeting the Milan criteria who undergo hepatectomy.

Key words *LIN28*, hepatocellular carcinoma, hepatectomy, the Milan criteria, prognosis

Hepatocellular carcinoma (HCC) is the fifth most common tumor type and the third most common cause of cancer death worldwide, with the majority of cases occurring in East Asia and sub-Saharan Africa[1]. Because of varied etiologies, including viral hepatitis, alcohol abuse, and metabolic syndrome, HCC is a highly heterogeneous disease with complex carcinogenesis[2]. Surgical resection or liver transplantation offers the chance of a cure, but only 30%–40% of HCC patients are eligible for curative treatments. The shortage of organs for transplantation has prompted liver surgeons to perform hepatectomy as the first-choice therapy for HCC patients, even those who meet the Milan criteria. Despite clear advances made in molecular targeted therapy for HCC since 2008[3], favorable effects of this therapy on the survival of patients with advanced or recurrent disease have not been observed.

Comprehensive understanding of the molecular mechanisms of liver cancer stem cells may yield great advances in liver cancer targeted therapy. *LIN28*, a cancer stem cell marker, was first identified as an

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important regulator for embryonic development timing in the nematode *Caenorhabditis elegans*[4,5]. It is also an RNA-binding protein involved in maintaining the pluripotency of embryonic stem cells. In mouse embryonic stem cells, inhibition of *LIN28* leads to decreased cell proliferation, whereas up-regulation of *Lin28* results in accelerated cell differentiation[6]. In embryonic stem cells, *LIN28* selectively blocks let-7 precursor from being processed to mature microRNA[7,8].

Recent published work by Yu *et al.*[6] has shown that *LIN28* and three other stem cell markers, OCT4, NANOG, and SOX2, are sufficient in reprogram human somatic cells to pluripotent stem cells, which further confirms the important role of *LIN28* in stem cells. In addition, *LIN28* mediates the post-translational expression of OCT4 by directly binding to its mRNA in human embryonic stem cells[9]. OCT4, NANOG, and SOX2 are not only important embryonic stem cell regulators; they are also critical cancer stem cell markers for malignancies[10,11]. Our previous study showed *OCT4* and *SOX2* were overexpressed in 136 HCC specimens and related with aggressive behavior as defined by large tumor size, vascular invasion, and poor survival in patients who underwent partial hepatectomy[12]. The tight relationship between *LIN28* and *OCT4* suggests that *LIN28* might be also a marker for HCC patients. However, few data on this subject are available. In this study, we performed reverse transcription-polymerase chain reaction (RT-PCR) on *LIN28* in a series of 129 HCC cases. We investigated whether *LIN28* was a biomarker for HCC and whether *LIN28* expression was associated with poor outcome in HCC. To determine the specificity of *LIN28* in tumor pathology, we also examined *LIN28* in 20 normal non-cirrhotic liver tissues.

Materials and Methods

Patients and sample collection

For this study, we collected 129 pairs of HCC tissues and adjacent non-tumorous liver tissues from patients treated between January 2001 and December 2006 at the Department of Hepatobiliary Surgery, Sun Yat-sen Cancer Center. Patients who met the following criteria were eligible for this study: (1) no prior anticancer treatment, (2) no evidence of concomitant extrahepatic disease, (3) no simultaneous use of local treatment modalities (i.e., radiofrequency ablation or microwave ablation), and (4) underwent curative resection, which was defined as complete removal of the tumor without macroscopic evidence of residual disease[13]. The enrolled patients consisted of 114 men (88.4%) and 15 women (11.6%), with a median age of 50 years (range, 26–79 years). Tumor size ranged from 2.0 to 19.5 cm, with a median of 6.0 cm. One patient was seropositive for both hepatitis C virus (HCV) antibody and hepatitis B surface antigen (HBsAg). A total of 114 patients (88.4%) was HBsAg-positive. All tumors were histologically diagnosed as HCC, with 11 Edmondson-Steiner grade I cases, 65 grade II cases, 40 grade III cases, and 13 grade IV cases. The tumor stages were classified according to the 7th edition American Joint Committee on Cancer (AJCC) TNM classification. Sixty-eight cases were classified as stage I, 38 as stage II, and 23 as stage III. Twenty normal non-cirrhotic liver tissues, which were from patients with liver hemangioma or focal nodular hyperplasia, were included as controls. All specimens were obtained immediately after surgical resection, snap frozen in liquid nitrogen, and stored at −80℃ until processing. The study was approved by the Ethics Committee of Sun Yat-sen University Cancer Center, and written informed consent was obtained from each patient before surgery.

RT-PCR

Each sample was evaluated in a RT-PCR procedure carried out as described in a previous paper[14]. Briefly, total RNA was isolated from liver tissues with Trizol-A+ agent. RNA was treated with DNase I (Invitrogen Inc., Carlsbad, CA) to remove any DNA contamination and quantified by spectrophotometry. Reverse transcription was performed with the SuperScript RT kit (Promega Inc., Madison, WI) according to the manufacturer's instructions, and the resultant cDNA templates were subjected to PCR amplification. To verify cDNA integrity, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression was analyzed as the housekeeping gene. The primer sets were 5′-CGGGCATCTGTAAGTGGTTC-3′ (forward) and 5′-CAGACCCTTGGCTGACTTCT-3′ (reverse) for *LIN28*[9], and 5′-ACAGTCCATGCCATCACTGCC-3′ (forward) and 5′-GCCTGCTCCACCACCTTCTTG-3′ (reverse) for *GAPDH*. PCR was performed for 35 cycles using the following parameters: denaturation (94℃, 30 s), annealing (60℃, 30 s), and extension (72℃, 30 s). The final products were separated using 2% agarose gel electrophoresis and visualized after ethidium bromide staining. Each reaction was performed twice independently.

Follow-up

The study was censored on June 30, 2010. The follow-up duration was defined as the interval between the date of resection and the date of death or the last follow-up. The median follow-up time was 31.0 months, ranging from 2.0 to 81.5 months. All patients were followed up every 1–3 months in the first year and every 3–6 months thereafter. The follow-up protocol included

physical examination, serum α-fetoprotein (AFP) level, chest X-ray, and abdominal ultrasonography. Computed tomography, magnetic resonance imaging, and/or positron emission tomography were performed when intrahepatic recurrence or distant metastasis was suspected. During follow-up, 70 (54.3%) of 129 patients developed intrahepatic recurrence or distant metastases, 49 (37.8%) died of cancer-related causes, and 4 (3.1%) died of other causes. Six patients (4.7%) were lost to follow-up.

Statistical analysis

Data analyses were carried out using the SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL). Chi-square test or Fisher's exact test was used to compare the relationship between *LIN28* expression and clinicopathologic parameters. Overall survival (OS) and recurrence-free survival (RFS) curves were generated by using the Kaplan-Meier method, and the difference between curves was assessed by log-rank test. Independent prognostic factors were analyzed by using the Cox multivariate proportional hazards regression model in a stepwise manner. All *P* values were 2-sided and a value of $P < 0.05$ was considered significant.

Results

High-frequency expression of *LIN28* in HCC tissues

Of the 129 paired HCC specimens, the positive rate of *LIN28* was significantly higher in HCC tissues than in the corresponding non-tumorous liver tissues (45.0% vs. 21.7%, $P = 0.020$). Of the 17 paired specimens in which *LIN28* was detected in both tumor tissues and non-tumorous tissues, 12 (70.6%) had a relatively higher density of *LIN28* in HCC tissues than in non-tumorous tissues. The expression of *LIN28* mRNA in normal non-cirrhotic liver tissues was undetectable (Figure 1).

Correlation of *LIN28* mRNA expression and clinicopathologic parameters

To further characterize *LIN28* in HCC, we identified the relationship of *LIN28* expression with conventional clinicopathologic parameters, including patient gender, age, HBsAg status, gamma-glutamyl-transferase (GGT) levels, Child-Pugh classification, serum AFP level, tumor size, tumor number, tumor capsule status, vascular invasion, cirrhosis, Edmondson–Steiner grade, and TNM stage. As shown in Table 1, *LIN28* mRNA expression was significantly associated with tumor size. Positive *LIN28* expression was more frequent in patients with large tumors ($P = 0.010$). No relationship was found between the expression of *LIN28* and other clinicopathologic variables.

Prognosis of HCC subtypes defined by mRNA level of *LIN28*

Next, we performed univariate and multivariate analyses to determine whether *LIN28* expression is associated with poor prognosis. The association of OS and RFS with clinicopathologic variables in 129 cases of HCC with or without *LIN28* expression is shown in Table 2. Tumor size > 5 cm ($P < 0.001$), multiple tumors ($P = 0.006$), presence of vascular invasion ($P < 0.001$), and TNM stage II/III ($P < 0.001$) were significant risk factors associated with unfavorable OS in patients with detectable *LIN28* expression. Tumor size > 5 cm ($P < 0.001$), multiple tumors ($P = 0.001$), presence of vascular invasion ($P < 0.001$), absence of tumor capsule ($P = 0.018$), Edmondson-Steiner grade III/IV ($P = 0.025$), and TNM stage II/III ($P < 0.001$) were significant risk factors associated with unfavorable RFS in patients with detectable *LIN28* expression. The difference in 5-year OS between the negative *LIN28* group and the positive *LIN28* group was nearly significant (54.7% vs. 41.8%, $P = 0.067$) (Figure 2A), and the difference in RFS between the two groups was not significant (59.4% vs. 27.2%,

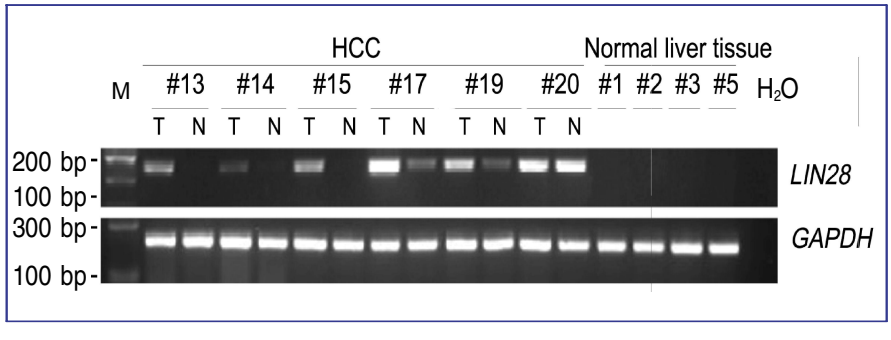

Figure 1. **Analysis of *LIN28* expression in liver tissues.** Representative *LIN28* mRNA expression in hepatocellular carcinomas (T), matching non-tumorous tissues (N), and normal non-cirrhotic human liver tissues was detected by reverse transcription-polymerase chain reaction. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an internal control. Products of 191 bp and 266 bp were expected for *LIN28* and *GAPDH* transcripts, respectively.

Table 1. Association between *LIN28* mRNA expression and clinicopathologic features of hepatocellular carcinoma (HCC)

Characteristic	No. of patients	*LIN28* mRNA expression [cases (%)]	*P* [c]
Gender			0.130
Female	15	4 (26.7)	
Male	114	54 (48.2)	
Age (years)[a]			0.937
⩽ 50	64	29 (45.3)	
> 50	65	29 (44.6)	
HBsAg status			0.336
Negative	15	5 (33.3)	
Positive	114	53 (46.5)	
AFP (μg/L)			0.619
⩽ 400	77	36 (46.8)	
> 400	52	22 (42.3)	
Cirrhosis			0.497
Absent	13	7 (53.8)	
Present	116	51 (44.0)	
Child-Pugh classification[b]			0.888
A	114	51 (44.7)	
B	15	7 (46.7)	
Tumor size (cm)			0.010
⩽ 5	56	18 (32.1)	
> 5	73	40 (54.8)	
Multiple tumors			0.722
No	91	40 (44.0)	
Yes	38	18 (47.4)	
Tumor capsule			0.297
Complete	30	11 (36.7)	
Incomplete	99	47 (47.5)	
Vascular invasion			0.980
No	98	44 (44.9)	
Yes	31	14 (45.2)	
Edmondson-Steiner grade			0.765
I/II	76	35 (46.1)	
III/IV	53	23 (43.4)	
TNM stage			0.105
I	68	26 (38.2)	
II/III	61	32 (52.5)	

HbsAg, hepatitis B virus surface antigen; AFP, α-fetoprotein. [a]Patients were divided according to their median age. [b]No patients with Child-Pugh C were included. [c]Chi-square or Fisher´s exact test.

P = 0.104) (Figure 2B).

Stratified univariate and multivariate analysis

An increasing number of HCC patients who meet the Milan criteria and have preserved hepatic function undergo hepatectomy and experience long-term RFS, similar to that of liver transplantation[15,16]. Some of those patients, however, may experience early recurrence following hepatectomy. We therefore tried to define the role of *LIN28* in identifying patients meeting the Milan criteria, which is currently widely accepted in the selection of candidates for transplantation [17], and who have a high risk of recurrence after resection. A total of 53 patients met the Milan criteria. Among them, 44 patients presented with one lesion, 4 with two lesions, and 5 with three lesions. For those 53 patients, significant associations were found between *LIN28*

Table 2. Univariate prognostic analysis of overall survival (OS) and recurrence-free survival (RFS) rates for HCC patients after hepatectomy

Characteristic	No. of patients	OS rate (%)		*P*	RFS rate (%)		*P*
		3-year	5-year		3-year	5-year	
Gender				0.118			0.047
Female	15	86.7	62.6		62.7	62.7	
Male	114	54.7	45.2		39.2	37.4	
Age (years)				0.352			0.165
⩽ 50	64	56.2	46.2		42.5	39.0	
> 50	65	61.8	48.5		44.9	44.9	
HBsAg status				0.181			0.071
Negative	15	84.8	56.6		63.7	63.7	
Positive	114	55.6	46.6		40.9	39.2	
AFP (μg/L)				0.595			0.830
⩽ 400	77	55.4	47.6		42.4	39.1	
> 400	52	62.4	58.7		45.8	45.8	
Cirrhosis				0.208			0.904
Absent	13	74.1	74.1		44.9	44.9	
Present	116	57.2	45.8		43.4	41.7	
Child-Pugh classification				0.713			0.257
A	114	59.0	47.0		46.4	44.6	
B	15	58.8	58.8		16.5	16.5	
Tumor size (cm)				<0.001			<0.001
⩽ 5	56	78.4	73.7		65.0	65.0	
> 5	73	45.3	32.1		27.8	25.5	
Multiple tumors				0.006			0.001
No	91	64.7	57.5		52.1	52.1	
Yes	38	45.8	25.8		23.3	18.8	
Tumor capsule				0.078			0.018
Complete	30	71.9	64.5		64.5	64.5	
Incomplet	99	55.1	42.6		37.4	35.5	
Vascular invasion				<0.001			<0.001
No	98	68.6	59.7		52.9	50.9	
Yes	31	27.7	13.9		13.6	13.6	
TNM stage				<0.001			<0.001
I	68	72.9	65.7		59.4	59.4	
II/III	61	43.3	29.6		25.2	22.4	
Edmondson-Steiner grade				0.060			0.025
I/II	76	66.6	49.8		51.8	48.8	
III/IV	53	48.3	43.8		32.8	32.8	
LIN28 mRNA expression				0.067			0.104
Negative	71	62.3	54.7		46.8	46.8	
Positive	58	47.8	41.8		40.4	33.7	

Abbreviations as in Table 1.

expression and OS (P = 0.007) and RFS (P < 0.001) (Figure 3). The 5-year OS rates were 87.6% versus 49.1% and the 5-year RFS rates were 68.3% versus 33.1% in the *LIN28*-negative and *LIN28*-positive groups, respectively. The association of OS and RFS with clinicopathologic features in HCC patients meeting the Milan criteria is shown in Table 3. Adjusting for other prognostic variables in the multivariate Cox model, *LIN28* remained an independent negative prognostic factor for both OS and RFS (Table 4). *LIN28*-positive patients meeting the Milan criteria had shorter OS [hazard rate (HR), 7.039; 95% confidence interval (CI),

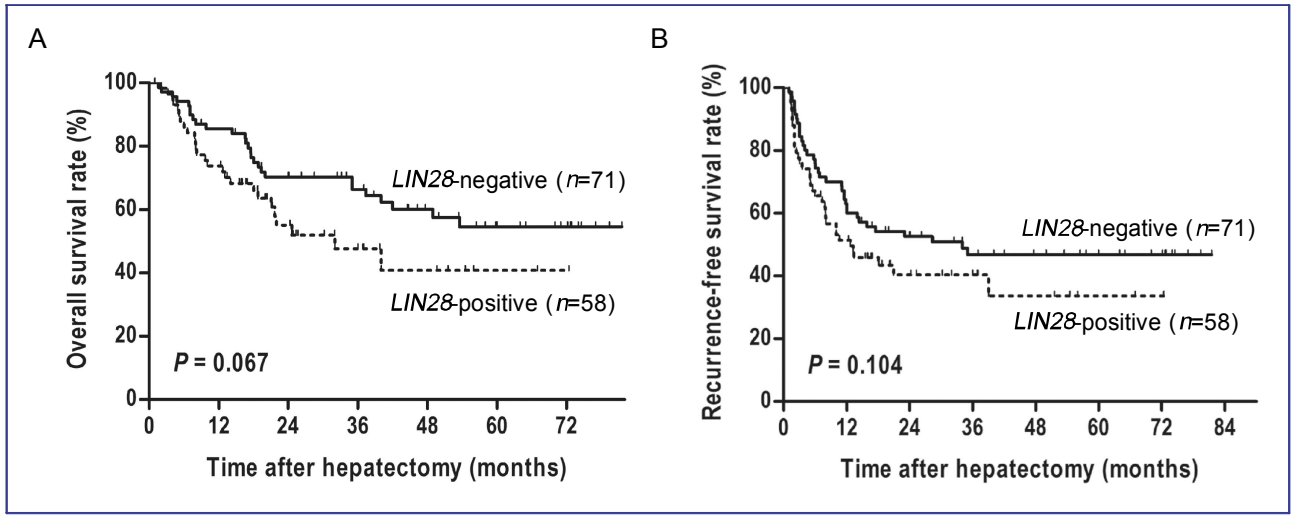

Figure 2. **Kaplan-Meier survival curves of 129 HCC patients with or without *LIN28* mRNA expression.** A, overall survival (log-rank, $P = 0.067$); B, recurrence-free survival (log-rank, $P = 0.104$).

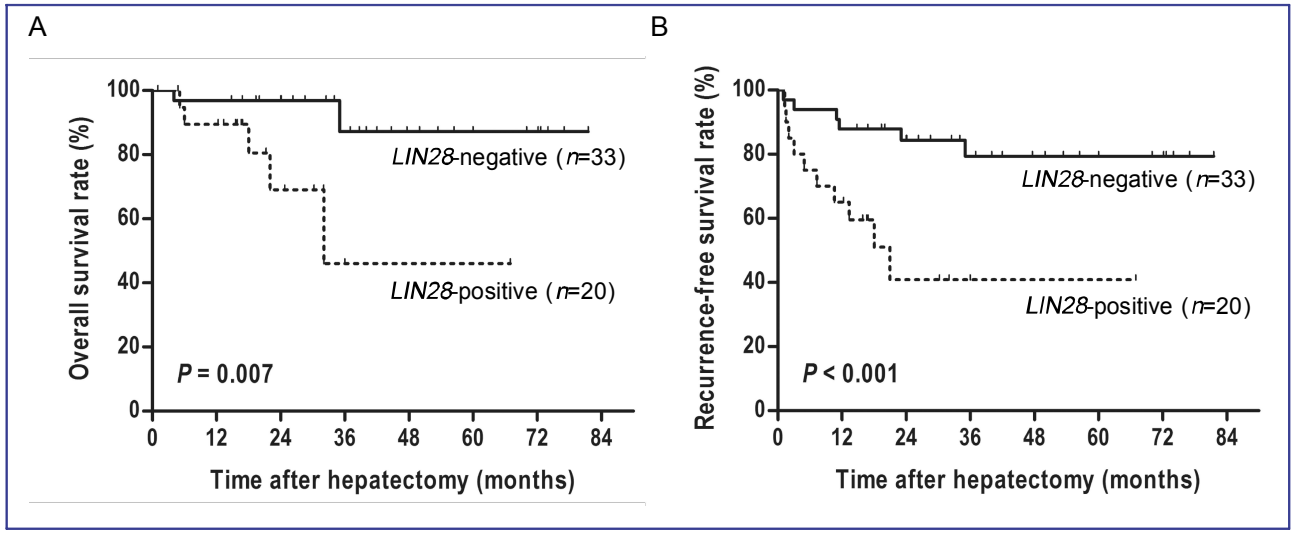

Figure 3. **Kaplan-Meier survival curves of HCC patients meeting the Milan criteria with or without *LIN28* mRNA expression.** A, overall survival (log-rank, $P = 0.007$); B, recurrence-free survival (log-rank, $P < 0.001$).

1.427–34.724; $P = 0.017$] and RFS (HR, 5.518; 95% CI, 1.738–17.520; $P = 0.004$) than *LIN28*-negative patients. In addition, no significant associations of *LIN28* and OS and RFS were observed in the 76 patients who did not meet the Milan criteria ($P = 0.582$ and $P = 0.750$, respectively).

Discussion

Our study showed that *LIN28* was expressed at a higher frequency in HCC samples than in non-HCC samples. Furthermore, *LIN28* expression related with poor survival of HCC patients who met the Milan criteria but not for those who did not meet the Milan criteria.

In recent years, numerous studies have pointed to the fact that malignancies are derived from stem cells. Similar to most solid tumors, HCC are believed to contain cancer stem-like cells that initiate tumorigenesis, prompt tumor recurrence, and confer resistance to chemotherapy [18-20]. *LIN28* has been reported to be highly expressed in several solid tumors, including tumors of the ovaries [21], testicular germ cells [22], and colon [23]. Overexpression of *LIN28* was associated with an increase in metastatic ability and poor prognosis in colon cancer[23]. Further studies demonstrated that *LIN28* plays a critical role in maintaining the undifferentiated state of

Table 3. Univariate analysis of prognostic factors for OS and RFS in the 53 HCC patients meeting the Milan criteria

Characteristic	No. of patients	OS rate (%)		*P*	RFS rate (%)		*P*
		3-year	5-year		3-year	5-year	
Gender				0.155			0.055
Female	8	100.0	100.0		100.0	100.0	
Male	45	72.9	72.9		60.0	60.0	
Age (years)				0.608			0.602
⩽ 50	25	76.5	76.5		66.7	66.7	
> 50	28	79.8	79.8		67.2	67.2	
HBsAg status				0.718			0.561
Negative	8	83.3	83.3		83.3	83.3	
Positive	47	77.6	77.6		65.0	65.0	
AFP (μg/L)				0.417			0.231
⩽ 400	34	71.0	71.0		60.4	60.4	
> 400	19	88.4	88.4		77.5	77.5	
Cirrhosis				0.362			0.341
Absent	4	100.0	100.0		50.0	50.0	
Present	49	75.4	75.4		67.5	67.5	
Child-Pugh classification				0.734			0.289
A	46	79.1	79.1		68.2	68.2	
B	7	75.0	75.0		57.1	57.1	
Tumor size (cm)				0.800			0.841
⩽ 3.5	28	79.2	79.2		67.5	67.5	
> 3.5	25	77.7	77.7		65.9	65.9	
Multiple tumors				0.076			0.006
No	44	82.1	82.1		74.3	74.3	
Yes	9	60.0	60.0		33.3	33.3	
Tumor capsule				0.125			0.103
Complete	19	94.7	94.7		82.1	82.1	
Incomplete	34	68.2	68.2		57.5	57.5	
Vascular invasion				0.465			0.371
No	49	78.9	78.9		68.1	68.1	
Yes	4	66.7	66.7		50.0	50.0	
TNM stage				0.142			0.043
I	43	81.5	81.5		75.1	75.1	
II	10	65.6	65.6		45.4	45.4	
Edmondson-Steiner grade				0.830			0.549
I/II	34	75.9	75.9		66.3	66.3	
III/IV	19	80.0	80.0		63.2	63.2	
LIN28 mRNA expression				0.007			<0.001
Negative	33	87.6	87.6		79.4	79.4	
Positive	20	49.1	49.1		40.2	40.2	

Abbreviations as in Table 1.

embryonal testicular germ cell tumors, and presumably upstream of OCT3/4 and NANOG[24]. Investigators have recently reported that *LIN28* homolog B (*LIN28B*) is overexpressed in HCC and associated with advanced stage[25,26]. However, *LIN28* itself was not evaluated in the studies, and with a very limited number of primary HCCs, the predictive value of *LIN28* for HCC was not well established [25,26]. Our previous study indicated that OCT4 and SOX2 could be used as independent predictors for HCC patient outcome in PCR analysis [12]. Thus, RT-PCR analysis was performed on 129 pairs of matched HCC and non-tumorous tissues, as well as 20

Table 4. Multivariate analysis of factors contributing to OS and RFS for HCC patients meeting the Milan criteria

Characteristic	OS		RFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Gender	—	—	2.893 (0.037–6.991)	0.976
Multiple tumors	3.381 (0.766–14.916)	0.108	3.567 (0.504–25.449)	0.203
TNM stage	—	—	0.762 (0.106–5.473)	0.787
LIN28 mRNA expression	7.039 (1.427–34.724)	0.017	5.518 (1.738–17.520)	0.004

HR, hazard ratio; CI, confidence interval.

normal liver tissues to investigate *LIN28* expression and to explore its potential clinical value in HCC patients following resection.

In this study, we confirmed that expression of *LIN28* mRNA was markedly increased in HCC tissues compared to adjacent non-tumorous liver tissues but was undetectable in normal non-cirrhotic liver tissues. These results suggest that aberrant expression of *LIN28* may be involved in carcinogenesis, which is consistent with findings from previous studies[25,26]. Viswanathan *et al.*[26] showed that *LIN28B* overexpression was associated with high serum AFP level and advanced stage in 89 HCV-related HCC patients. In contrast, tumor size was the only factor related with *LIN28* expression in our series. The distinct disease background may help to explain the discrepancy in the two studies, but both studies show that *LIN28* or its homolog is associated with aggressive behavior in cancer cells, which can be characterized by high AFP level, advanced stage, and large tumor size[27]. Moreover, a recently published study showed that poorly differentiated, aggressive human tumor cells have an embryonic stem cell-like gene expression signature[28]. We presumed that a highly rich population of cancer stem cells were present in the large tumor size group (⩽5 cm) compared to the small tumor size group (⩽5 cm), leading to increased *LIN28* expression in patients with large tumors.

HCC patients with well-preserved liver function who meet with the Milan criteria have three potential curative therapeutic options: partial hepatectomy, local ablation, and liver transplantation. However, all three options have their relative advantages and disadvantages[29,30]. It should be noted that when resection is feasible, local ablation is not recommended due to a high local recurrence rate. This was confirmed by a recently published randomized controlled clinical trial[31]. On account of organ shortage and lifelong immunosuppression, increasing studies support the hepatectomy as the first-choice therapy in areas where HCC prevalence is high[15,16,32]. However, some patients may develop intrahepatic recurrence after curative resection. Thus, predictive markers will be helpful to identify patients meeting the Milan criteria who are at high risk for recurrence after resection and may be eligible for transplantation. To the best of our knowledge, there is only one study related to specific biomarkers that predict prognosis after resection for HCC patients meeting the Milan criteria [33]. Therefore, more predictive biomarkers are critical for the management of these HCC patients.

In the present study, we showed that *LIN28* was a useful marker to predict poor prognosis both in univariate and multivariate analyses for patients who met the Milan criteria and underwent hepatectomy. Although extended transplantation criteria has been receiving increased attention[34], the Milan criteria is still the most acceptable criteria for liver transplantation[35]. It is common that HCC patients meeting the Milan criteria undergo hepatectomy as the first-choice therapy in East Asia. Therefore, a strategy of more detailed follow-ups and future salvage transplantation for those patients with higher risk is critically needed for the areas where HCC is prevalent. *LIN28* will be helpful to identify high-risk HCC patients and provide information about how these patients will benefit from hepatectomy. In addition, establishing the predictive value of *LIN28* could alleviate the current shortage of donor livers. A favorable outcome predicted early following radical resection could also ensure patients undergo low-risk surgery and incur a low economic burden because transplantation is not involved. Moreover, these data provide new insights into the role of *LIN28* specifically in early stage HCC and not over the whole course of disease development. Nevertheless, since this study primarily enrolled hepatitis B virus–dependent HCC patients (88.4%), further studies are needed to investigate the relationship between *LIN28* expression and prognosis in human HCC attributed to other etiologies.

There are several limitations in this study. Our study was restricted to a solitary center and was based on an mRNA-level test. Also, the sample size of the subgroup of patients who met the Milan criteria was relatively small. A large scale, multi-center study is needed to confirm our results.

Our study showed that *LIN28* was expressed at a

high frequency in HCC, similar to previous studies [25,26]. However, our study differed from these previous studies in several aspects. First, in other studies, the target gene was not in *LIN28* itself but *LIN28* homolog. Second, the previous studies were performed on tissues with little or no information on the value of predicting prognosis in HCC patients after hepatectomy. In our study, we investigated *LIN28* in 129 paired HCC tissues and explored its predictive value on OS and RFS in patients following curative resection. Unexpectedly, the association between *LIN28* expression and outcome failed to reach statistical significance. We further determined the predictive value of *LIN28* in patients who met the Milan criteria because there are no data on *LIN28* in this specific population in the literature. Our results showed that *LIN28* is a valuable biomarker to identify individuals meeting the Milan criteria who have a high risk of recurrence. Further studies should include patients who have undergone liver transplantation to determine if *LIN28* has any predictive value after transplantation.

Conclusion

In conclusion, the present study showed that *LIN28* was overexpressed in HCC tissues. *LIN28* may be an independent negative predictor for OS and RFS for patients meeting the Milan criteria, but not for all HCC patients. Examination of *LIN28* may help to identify high-risk HCC patients who meet the Milan criteria and underwent hepatectomy.

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