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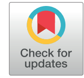
Original Article

Identification of Expression Pattern and Clinical Significance of the Small Cajal Body-specific RNA SCARNA16 in Hepatocellular Carcinoma



Sitong Zhang[1#], Yuan Ding[1#], Zhongquan Sun[1], Yao Ge[2], Yanjie Li[1], Xin Han[1], Qianhui Xu[1], Liuzhi Zhou[1], Yining Chen[1], Hao Xu[1], Yang Bai[1], Chang Xu[1], Hao Ding[1], Sheng Yan[1*] and Weilin Wang[1*]

[1]Department of Hepatobiliary and Pancreatic Surgery, The Second Affiliated Hospital, Zhejiang University School of Medicine, Key Laboratory of Precision Diagnosis and Treatment for Hepatobiliary and Pancreatic Tumor of Zhejiang Province, Research Center of Diagnosis and Treatment Technology for Hepatocellular Carcinoma of Zhejiang Province, Clinical Medicine Innovation Center of Precision Diagnosis and Treatment for Hepatobiliary and Pancreatic Disease of Zhejiang University, Clinical Research Center of Hepatobiliary and Pancreatic Diseases of Zhejiang Province, Zhejiang University Cancer Center, Hangzhou, Zhejiang, China; [2]Key Laboratory of Precision Diagnosis and Treatment for Hepatobiliary and Pancreatic Tumor of Zhejiang Province, Research Center of Diagnosis and Treatment Technology for Hepatocellular Carcinoma of Zhejiang Province, Clinical Medicine Innovation Center of Precision Diagnosis and Treatment for Hepatobiliary and Pancreatic Disease of Zhejiang University, Clinical Research Center of Hepatobiliary and Pancreatic Diseases of Zhejiang Province, Zhejiang University Cancer Center, Hangzhou, Zhejiang, China

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Abstract

Background and Aims: For high morbidity and mortality, hepatocellular carcinoma (HCC) becomes a major health issue worldwide. Nowadays, numerous non-coding RNAs (ncRNAs) are known to regulate the occurrence and pathogenesis of tumors. Some ncRNAs have also been developed as tumor biomarkers and therapeutic targets. However, the potential function of the small Cajal body-specific RNA (scaRNA) SCARNA16, a newly identified ncRNA, remains to be explored in HCC. ***Methods:*** In both HCC cell lines and specimens from 120 enrolled patients, the expression values of SCARNA16 were detected. We divided patients into SCARNA16 high and low expression subgroups, and then analyzed the difference of various clinical characteristics and prognosis data between subgroups. ***Results:*** Compared to paired controls, SCARNA16 was significantly down-regulated in HCC cell lines and clinical specimens ($p<0.01$). Besides, HCC patients with lower SCARNA16 expression commonly presented with larger and more tumor lesions, more vessel carcinoma emboli, more capsular invasion and higher TNM stages ($p<0.05$). Moreover, SCARNA16 expression was negatively correlated with postoperative prognosis of HCC patients in 5-year follow-up, including tumor-free survival (TFS) (median time of low vs. high subgroups: 14 vs. 48 months, $p=0.006$) and overall survival (OS) (median time of low vs. high subgroups: 39 vs. 52 months, $p=0.001$). Besides, SCARNA16 acted as an independent prognostic biomarker in TFS (hazard ratio [HR]: 0.578, 95% CI: 0.345–0.969, $p=0.038$) and OS (HR: 0.366, 95% CI: 0.178–0.752, $p=0.006$). ***Conclusions:*** Low expression patterns of SCARNA16 remarkably associated with severe clinical status and poor survival of patients, suggesting that SCARNA16 possesses potency as a novel biomarker for HCC.

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Introduction

Nowadays, primary hepatic carcinoma has been ranked as the fifth most common cancer along with the fourth largest cause of cancer-related mortality.[1] Indeed, the morbidity of liver cancer remains remarkably high around the world, especially in Eastern Asia.[2] Among those cases, hepatocellular carcinoma (HCC) accounts for approximately 90% of the primary liver cancer cases.[3] Although a lot of clinical therapies have been applied to treat HCC, the survival benefit to HCC patients is still limited for its high metastasis and relapse as well as fatality rates.[4] In view of the severe malignancy and heterogeneity of HCC, an individualized treat-

Keywords: Hepatocellular carcinoma; Non-coding RNA; SCARNA16; Clinical assessment; Biomarker.

Abbreviations: CB, Cajal bodies; CI, confidence interval; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; ncRNA, non-coding RNA; OS, overall survival; qRT-PCR, quantitative real-time polymerase chain reaction; RNP, ribonucleoprotein; scaRNA, small Cajal body-specific RNA; snoRNP, small nucleolar RNP; TFS, tumor-free survival.

[#]Contributed equally to this work.

[*]**Correspondence to:** Weilin Wang, Department of Hepatobiliary and Pancreatic Surgery, The Second Affiliated Hospital, School of Medicine, Zhejiang University, No. 88 Jiefang Road, Hangzhou, Zhejiang 310009, China. ORCID: https://orcid.org/0000-0001-9432-2649. Tel: +86-571-8778-3820, Fax: +86-571-8706-8001, E-mail: wam@zju.edu.cn; Sheng Yan, Department of Hepatobiliary and Pancreatic Surgery, The Second Affiliated Hospital, School of Medicine, Zhejiang University, No. 88 Jiefang Road, Hangzhou, Zhejiang 310009, China. ORCID: https://orcid.org/0000-0002-4153-3546. Tel: +86-571-8723-6688, Fax: +86-571-8723-6765, E-mail: shengyan@zju.edu.cn

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ment strategy is deemed to improve the prognosis of these patients. Apparently, it is particularly necessary to determine some novel suitable biomarkers, which can be used in clinical assessment and prognostic analysis of HCC patients.

In addition to the typically identified oncogenic and tumor suppressor genes, nowadays, more and more RNAs derived from noncoding regions within the genome (noncoding RNA, ncRNA) are ascertained to be extensively associated with tumorigenesis.[5,6] As generally demonstrated by many researches, ncRNAs could directly participate in plentiful intracellular process by regulating transcription and translation of corresponding proteins.[7] Among that, the small Cajal body-specific RNAs (scaRNAs), one special subset representing an ncRNA family derived from Cajal bodies, are commonly supposed to play vital roles in modifying other RNA family members, including mRNAs, tRNAs, rRNAs and snRNAs.[8] However, it was demonstrated that dysregulation of the scaRNA SCARNA2 could promote tumor development and chemotherapy resistance of colorectal cancer via facilitating EGFR and Bcl-2 protein expression.[9] Moreover, the expression pattern of sno/scaRNAs was successfully applied to characterize distinct molecular subtypes of multiple myeloma by Ronchetti's team,[10] and Chu *et al*.[11] also reported that the sno/scaRNAs expression profile could function as novel biomarkers and predict the clinical outcome of chronic lymphocytic leukemia patients. Thus, it is necessary to find the underlying tumor-related scaRNAs and explore its regulatory roles as well as clinical value in HCC.

SCARNA16, a newly identified scaRNA located in the Cajal body, has been demonstrated to directly participate in RNA processing.[8] However, its potential association with biological or clinical characteristics of malignant tumors remains unknown. In the present research, we aimed to explore the expression pattern of SCARNA16 in HCC cells as well as tissues. Then, we investigated its potential value in clinical assessment and prognostic analysis for HCC patients.

Methods

Cell culture

The human HCC cell lines, including Hep3B, PLC/PRF/5, SK-HEP-1 and Huh-7, and normal hepatocyte cell line QSG-7701 were used in this study. Cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Invitrogen, Waltham, MA, USA) with 10% fetal bovine serum (Gibco) and 1% streptomycin/penicillin (Gibco) under an atmosphere of 5% CO_2 at 37°C.

Patient enrollment and specimen collection

This retrospective research study enrolled 120 consecutive HCC patients from January 2013 to December 2014, who underwent hepatectomy and were diagnosed with HCC according to histological tests. Any patient with history of preoperative radiotherapy, chemotherapy and immunotherapy before hepatectomy was excluded. Informed consent was obtained from all enrolled patients. The clinical characteristic and basic information of patients were collected from the hospital case system. HCC and adjacent liver specimens were acquired from resected liver tissues and quickly stored in −80°C refrigerator for future examination.

Postoperative follow-up

Within 5 years after hepatectomy, the valid survival and tumor recurrence information was obtained from enrolled HCC patients by telephone or periodic review in hospital. The time interval of tumor-free survival was recorded from the date of hepatectomy to tumor recurrence, distant metastasis, or individual death. The measurement end point of overall survival (OS) was individual death or the last follow-up date. All patients and their family members were blind to the grouping of this research, and the experimenters responsible for postoperative follow-up were blind to clinical data of the enrolled patients.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) analysis

With use of Trizol reagent (Invitrogen, Carlsbad, CA, USA) following manufacturer's instructions, RNA extraction was performed for collected cells and tissues. Then, total RNA was assessed by Nanodrop 2000 spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA) and then it was reverse transcribed to cDNA using a Reverse Transcriptase kit (Vazyme, Nanjing, China). To calculate the relative expression values of SCARNA16, cDNA samples mixed with SYBR Green Master ROX (Roche, Basel, Switzerland) were subjected to qRT-PCR according to the manufacturer's instructions. GAPDH was tested as endogenous control, and quantitation of SCARNA16 expression was calculated by the $2^{-\Delta\Delta Ct}$ formula. All samples were tested in triplicate. The primer sequences included: SCARNA16: 5′-GGGAAAGGCTCCTGTGTTG-3′ (forward), 5′-CTTTAGGTCAGGGTTGGGC-3′ (reverse); GAPDH: 5′-CAGGAGGCATTGCTGATGAT-3′ (forward), 5′-GAAGGCTGGGGCTCATTT-3′ (reverse).

Statistical analysis

Statistical software package SPSS19.0 (IBM Corp., Armonk, NY, USA) was used to accomplish all statistical analysis. Quantitative variables were assessed by Student's *t*-test. Chi-square test and Fisher's exact test were applied to analyze the correlations between SCARNA16 expression and clinical characteristics. Tumor-free survival (TFS) and OS data were evaluated by the Kaplan-Meier method and Log-rank test. Cox proportional hazards regression models were used to assess the independent influence of each index, and the index with p of less than 0.1 in the univariate Cox model was entered into the multivariate Cox model. A p value of less than 0.05 was considered to be statistically significant.

Ethical statement

This study was conducted according to the ethical guidelines of the Helsinki Declaration as revised in 2013. All participants signed the written informed consent form. Ethical approval was obtained from the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University. All data were analyzed anonymously and identified prior to analysis.

Results

SCARNA16 expression patterns in HCC

To explore the potential functional role of SCARNA16 in HCC, the SCARNA16 expression values were initially detected in HCC cell lines and the normal hepatocyte cell line QSG-7701. As shown in Figure 1, SCARNA16 expression

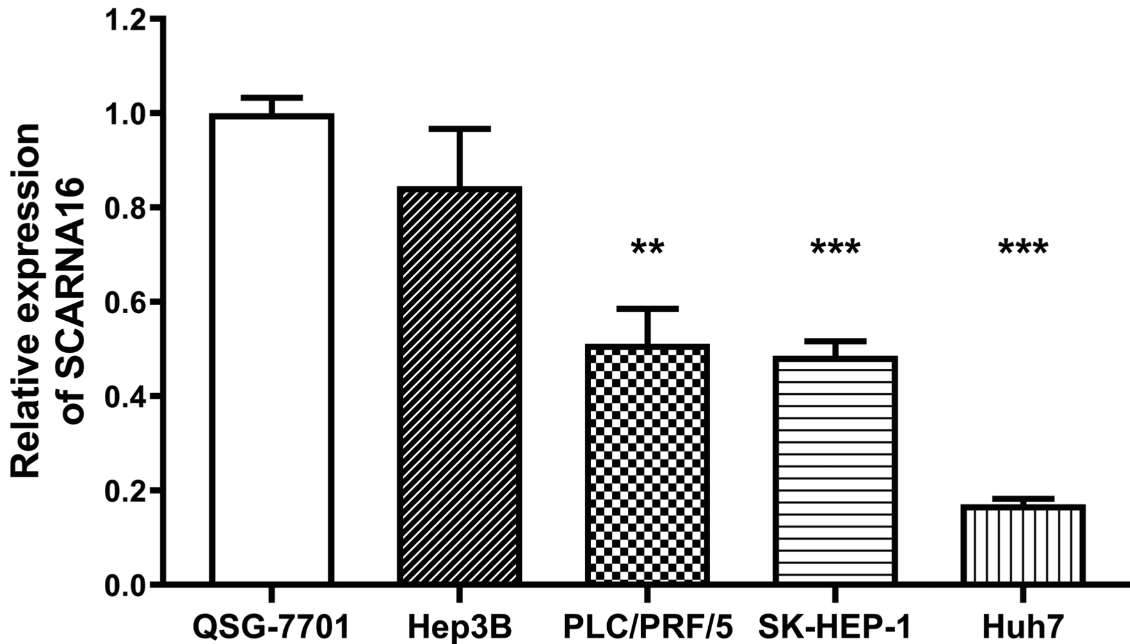

Fig. 1. Relative SCARNA16 expression levels in HCC and normal hepatocyte cell lines. Recognizing QSG-7701 as the normal control, SCARNA16 universally showed significant down-regulated expression features in HCC cell lines, including PLC/PRF/5 (p=0.004), SK-HEP-1 (p<0.001) and Huh-7 (p<0.001). Relative expression = $2^{-\triangle\triangle Ct}$, $-\triangle\triangle Ct$ = ($Ct_{GAPDH}-Ct_{SCARNA16}$) of HCC cell lines − ($Ct_{GAPDH}-Ct_{SCARNA16}$) of QSG-7701. **p<0.01, ***p<0.001. HCC, hepatocellular carcinoma.

values of several HCC cells were significantly lower than that of normal hepatocytes (p<0.05; Fig. 1). Recognizing QSG-7701 as the normal control, the relative expression levels of SCARNA16 in different HCC cell lines were 0.845 (Hep3B), 0.511 (PLC/PRF/5, p<0.01), 0.485 (SK-HEP-1, p<0.001) and 0.171 (Huh-7, p<0.001), respectively.

To further verify the abnormal expression patterns of SCARNA16 were detected in HCC, HCC and related adjacent liver specimens from HCC patients. In Figure 2A, the results revealed that SCARNA16 expression of HCC tissues was remarkably lower than that of adjacent liver tissues (p=0.002; Fig. 2A). Among that, the down-regulated expression features of SCARNA16 were identified in 82.9% paired clinical HCC specimens (Fig. 2B). By reference to the verification results of both cell lines and tissues, it was demonstrated that SCARNA16 universally presented with decreased expression patterns in HCC and suggested it might function as a tumor biomarker in tumorigenesis.

Correlation between SCARNA16 expression and clinicopathological characteristics of HCC patients

According to the clinical values of SCARNA16 expression in

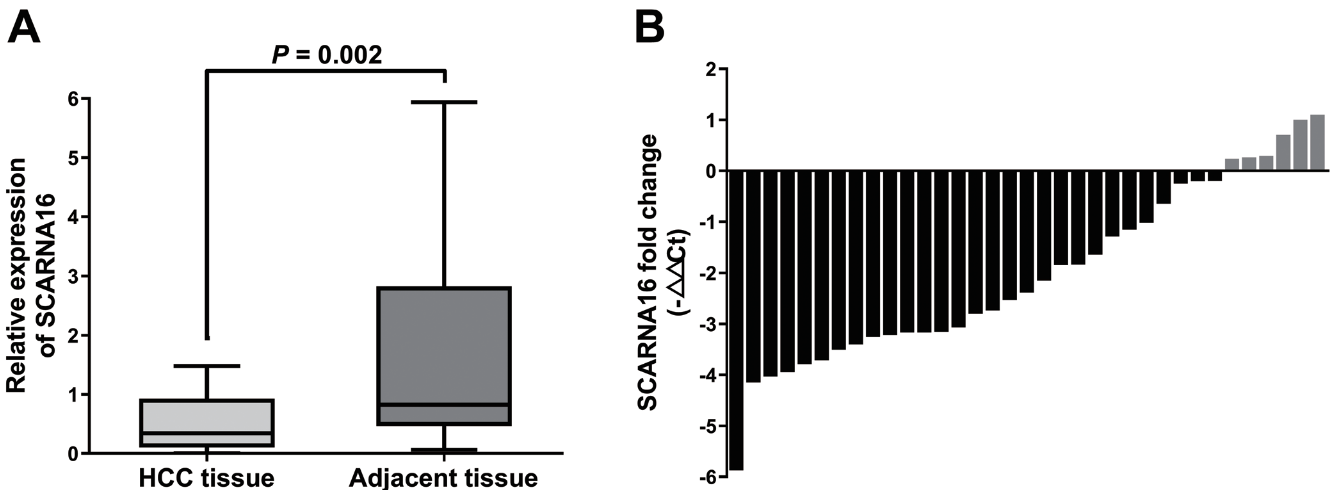

Fig. 2. Determination of SCARNA16 expression levels in clinical HCC and adjacent liver tissues. (A) Compared to paired adjacent liver tissues, the majority of clinical HCC tissues expressed remarkably lower levels of SCARNA16 (p=0.002). (B) The fold-change of SCARNA16 expression between HCC and adjacent liver tissues is shown in waterfall plot. Relative expression = $2^{-\triangle\triangle Ct}$, fold change ($-\triangle\triangle Ct$) = ($Ct_{GAPDH}-Ct_{SCARNA16}$) of HCC tissues − ($Ct_{GAPDH}-Ct_{SCARNA16}$) of adjacent liver tissue. HCC, hepatocellular carcinoma.

HCC, all patients were categorized into high and low expression subgroups. Based on this, the correlation between relative SCARNA16 expression levels and clinicopathological characteristics in HCC patients were further analyzed. Among all enrolled patients, there were 24 females (20.0%) and 96 males (80.0%). The median age of patients ranged from 26 to 82 years-old, with a median value of 55.1 years-old. In total, 101 out of 120 of the enrolled HCC patients were infected with the hepatitis B virus (HBV).

As shown in Table 1, HCC patients from the low SCARNA16 expression subgroup showed larger tumor lesions (p=0.001), higher incidences of vessel carcinoma emboli (p=0.006) and capsular invasion (p=0.023), and more severe TNM stage (p<0.001), revealing that low SCARNA16 expression was strongly associated with advanced clinical status of HCC. However, there was no distinct relevance found between SCARNA16 expression and α-fetoprotein value, HBV infection, liver cirrhosis or other basic liver function indexes (p>0.05; Table 1). Meanwhile, there was no significant difference in SCARNA16 expression between HCC patients with or without specific liver disease background, including HBV infection (p=0.664; Supplementary Fig. 1) or liver cirrhosis (p=0.313; Supplementary Fig. 2).

Association between SCARNA16 and TFS in HCC patients

As shown in Figure 3, the results of Kaplan-Meier analysis demonstrated that the high SCARNA16 expression group showed a more favorable TFS curve (median survival time of low vs. high expression subgroups: 14 vs. 48 months, p=0.006), indicating that the HCC patients with low SCARNA16 expression had relatively higher risk of tumor relapse. Furthermore, the independent risk impact of each characteristics on TFS was assessed using multivariate Cox proportional hazards regression analysis. As shown in Table 2, besides TNM stage, SCARNA16 expression also behaved as another independent risk factor for TFS of HCC patients (hazard ratio [HR]: 0.578, 95% confidence interval [CI]=0.345–0.969, p=0.038). Meanwhile, no impact of sex, age, HBV infection, liver cirrhosis or tumor size was found on the TFS of HCC patients.

Association between SCARNA16 and OS in HCC patients

Furthermore, to determine the impact of SCARNA16 expression on OS, the OS curves based on 5-year follow-up of HCC patients were plotted (Fig. 4). The results showed that HCC patients with relatively high SCARNA16 expression had favorable OS (median survival time of low vs. high expression subgroups: 39 vs. 52 months, p=0.001). Following, multivariate Cox proportional hazards regression analysis demonstrated that expression of SCARNA16 functioned as a risk factor for long-term survival of HCC patients after hepatectomy (HR: 0.366, 95% CI=0.178–0.752, p=0.006; Table 3), which was independent of HBV infection, liver cirrhosis and other liver function-related indexes.

Discussion

In the past decades, HCC remains a malignant disease with poor survival and high fatality rate, affecting individuals the world over.[12] Although there are several clinical treatments for HCC, including hepatectomy and chemotherapy, the mortality rate and long-term survival of HCC patients remains unsatisfactory.[13,14] Indeed, poor survival outcomes of HCC mainly account for its insidious tumor onset, heterogeneity and high risk of recurrence or metastasis.[15] With regard to these factors, nowadays, precise individual management, encompassing accurate disease diagnosis, tumor status evaluation and prognosis prediction, has attracted more and more importance.[16] Among that, an ideal tumor-related biomarker is necessary for clinical management of malignant tumors. On the one hand, the biomarker values in different individuals will provide effective evidence for doctors to assess the conditions. On the other hand, as the rapid progress of gene therapy and targeted therapy, some tumor markers have been developed into novel targets for cancer therapy, which becomes a major focus in field of cancer research.[17]

With application of genome sequencing technology, accumulating research findings have demonstrated that abnormal expression of various ncRNAs could directly cause complex processes of tumor progression, such as excessive cell proliferation, drug resistance, metastasis and immune escape.[18] The highly up-regulated in liver cancer ncRNA, HULC, recognized as a classic ncRNA detected in HCC, was confirmed to promote tumorigenesis via enhancing expression of the HMGA2 oncogene.[19] What's more, some scientific teams completed the transcriptome profiling analysis and then utilized specific ncRNAs' expression for assessment of clinical status.[20] Different from other traditional serum tumor biomarkers of HCC, like α-fetoprotein and glypican-3 (i.e. GPC3), tumor-related ncRNAs commonly act as the primary induction factors in HCC development rather than subsequent metabolites, which makes it have higher tumor-related specificity and more underlying values to be explored.

As one special subset of ncRNA, scaRNAs were first identified from Cajal bodies in 1984.[21] Generally, Cajal bodies are responsible in modification of ncRNAs prior to maturation via pseudouridylation and 2′-O-methylation.[22] Among this vital biological process, scaRNAs function as the necessary guides responsible for ncRNAs. Accumulating lines of evidence have demonstrated that scaRNA dysregulation during splicing could contribute to severe congenital heart diseases, neuromuscular disorders and various malignancies.[23–25] Due to the exploration of various cellular regulatory functions of scaRNAs, the mechanisms of scaRNA dysregulation in tumorigenesis have been well-studied, particularly in hematologic malignancies.[26,27] For instance, abnormal up-regulation of the scaRNA SCARNA22 functions in an oncogenic capacity by suppressing intracellular oxidative stress.[28] Moreover, scaRNAs have been reported to participate in regulation of the cell's reproductive capacity as part of telomerase RNA component, which protects the ends of chromosomes against enzyme telomerase degradation during continuous cell division.[29] Indeed, this characteristic determined that abnormal expression of some specific scaRNAs could induce excessive cell proliferation and malignancies.

Actually, the expression pattern and clinical relevance of scaRNAs in HCC have been rarely studied to date. In the present study, we have first performed an independent research regarding the relationship between scaRNA expression and liver cancer. We identified that the scaRNA SCARNA16 expression level was remarkably lower in HCC cell lines than in normal hepatocyte cells, and it was further confirmed in clinical HCC tissues of patients, suggesting that abnormal down-expression of SCARNA16 might play potential roles in the tumorigenesis of HCC. To determine the clinical significance and potential associated factors of SCARNA16 expression in HCC patients, various clinical characteristics and basic information were compared between HCC patients with low or high SCARNA16 expression level respectively. HCC patients with relatively low SCARNA16 ex-

Table 1. Correlation of SCARNA16 and clinical characteristics in HCC patients

Characteristics	Total	SCARNA16 expression		*p*
		Low, *n*=60	High, *n*=60	
Age (years)				0.334
<60	73	34 (0.57)	39 (0.65)	
≥60	47	26 (0.43)	21 (0.35)	
Sex				0.096
Female	24	8 (0.15)	16 (0.27)	
Male	96	52 (0.85)	44 (0.73)	
HBV				1.000
Positive	102	51 (0.85)	51 (0.85)	
Negative	18	9 (0.15)	9 (0.15)	
Cirrhosis				0.605
Present	73	38 (0.63)	35 (0.58)	
Absent	47	22 (0.37)	25 (0.42)	
ALT (U/L)				0.292
≤40	90	48 (0.80)	42 (0.70)	
>40	30	12 (0.20)	18 (0.30)	
Serum TBil (μmol/L)				0.536
≤17	88	46 (0.77)	42 (0.70)	
>17	32	14 (0.23)	18 (0.30)	
Serum albumin (g/L)				0.394
≥35	106	51 (0.85)	55 (0.92)	
<35	14	9 (0.15)	5 (0.08)	
AFP (ng/mL)				0.713
<200	62	30 (0.50)	32 (0.53)	
≥200	58	30 (0.50)	28 (0.47)	
Tumor diameter (cm)				0.001*
<5	35	9 (0.16)	26 (0.43)	
≥5	85	51 (0.84)	34 (0.57)	
Multiple lesions				0.029*
Absent	97	45 (0.74)	52 (0.87)	
Present	23	15 (0.26)	8 (0.13)	
Vessel carcinoma embolus				0.006*
Absent	89	38 (0.63)	51 (0.85)	
Present	31	22 (0.37)	9 (0.15)	
Microvascular invasion				0.768
Absent	112	56 (0.92)	56 (0.93)	
Present	8	4 (0.08)	4 (0.07)	
Capsular invasion				0.023*
Absent	74	31 (0.52)	43 (0.72)	
Present	46	29 (0.48)	17 (0.28)	
Differentiation				0.596
Low	65	34 (0.57)	31 (0.52)	
High/moderate	55	26 (0.43)	29 (0.48)	
TNM stage				<0.001*
I~II	74	33 (0.55)	41 (0.85)	
III~IV	36	27 (0.45)	9 (0.15)	

*$p<0.05$. Values are presented as *n* (proportion). AFP, α-fetoprotein; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; TBil, total bilirubin; TNM, tumor-node-metastasis.

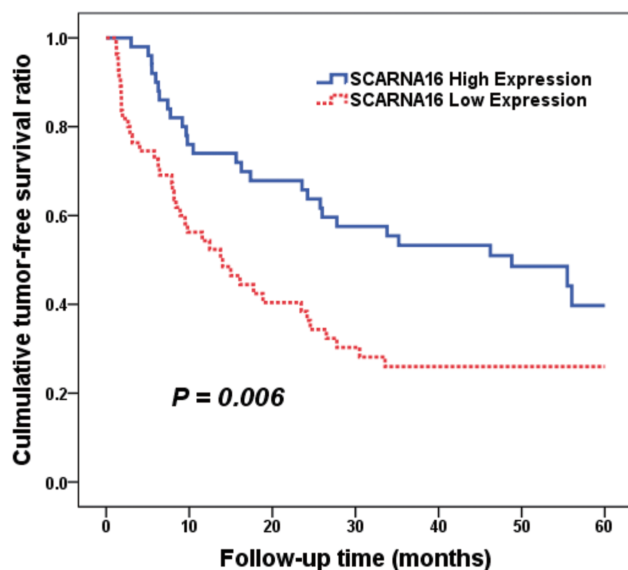

Fig. 3. Kaplan-Meier analysis in TFS of HCC patients with low and high SCARNA16 expression. During the postoperative 5-year follow-up, HCC patients with low SCARNA16 expression tended to have higher tumor relapse risk and shorter TFS time (p=0.006). TFS, tumor-free survival; HCC, hepatocellular carcinoma.

pression level manifested more advanced status of primary tumor lesions, including larger tumor size, more hepatic lesions and higher malignancy degrees, while SCARNA16 expression showed no significant correlation with neither basic liver function nor liver disease backgrounds of HCC patients.

In the cellular experiments, it was demonstrated that the down-regulated expression patterns of SCARNA16 tended to occur significantly more in hepatitis virus-associated HCC cell lines (PLC/PRF/5: HBV-related HCC cells; Huh7: hepatitis C virus [HCV]-related HCC cells). Meanwhile, considering that hepatitis virus infection and liver cirrhosis are another recoginized pathogenic factor of HCC, we considered whether the SCARNA16 expression level was affected by these liver disease backgrounds. Since none of enrolled patients was infected by HCV, we subdivided them according to their medical history of HBV infection or liver cirrhosis, and then demonstrated that SCARNA16 expression showed no remarkable difference among these subgroups, which supported that SCARNA16 expression was not remarkably influenced by the liver disease background of enrolled patients. Besides, down-regulation of SCARNA16 was found to be independently associated with both high postoperative recurrence rate and poor survival of HCC patients. To sum up, low SCARNA16 expression was shown to be an independent risk factor for tumor relapse as well as unfavorable survival outcomes in HCC patients. What's more, further investigations remain needed to illuminate the specific mechanism of SCARNA16 in HCC tumorigenesis. And, now, this finding about SCARNA16 and HCC patients is still a proof of concept, which needs more multicentric clinical data to be verified in following research studies.

However, there are some limitations in the present study that should be acknowledged. First, the postoperative follow-up of enrolled patients should be improved, to reduce random error from censored data. Besides, even though there was no significant difference in sex ratio between the SCARNA16 low and high expression groups, the sex ratio was almost 1:4 (female vs. male) in this study. Similarly, 90% of the enrolled HCC patients were infected by HBV, while no significant difference was found between HCC patients with or without HBV infection history. Indeed, HCC was largely attributable to chronic HBV infection in China, and almost 80% primary HCC patients had HBV infection history.[30] Thus, to reduce selection bias caused by differ-

Table 2. Univariate and multivariate analysis of TFS in HCC patients

Clinicopathological parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (<60 vs. ≥60 years)	0.788 (0.479–1.298)	0.350		
Sex (Female vs. Male)	1.478 (0.773–2.824)	0.237		
Hepatitis B (Negative vs. Positive)	1.254 (0.639–2.460)	0.510		
AFP (<200 vs. ≥200 ng/mL)	1.087 (0.670–1.764)	0.734		
Cirrhosis (Absent vs. Present)	1.283 (0.777–2.120)	0.331		
ALT (≤40 vs. >40 U/L)	0.718 (0.397–1.296)	0.271		
Serum TBil (≤17 vs. >17 μmol/L)	1.050 (0.615–1.792)	0.859		
Serum albumin (≥35 vs. <35 g/L)	1.425 (0.726–2.795)	0.303		
Tumor diameter (<5 vs. ≥5cm)	1.468 (0.861–2.505)	0.159		
Microvascular invasion (Absent vs. Present)	1.677 (0.759–3.703)	0.201		
Differentiation (High/moderate vs. Low)	1.137 (0.700–1.847)	0.605		
Multiple lesions (Absent vs. Present)	1.814 (1.031–3.190)	0.039	1.492 (0.738–2.766)	0.290
Capsular invasion (Absent vs. Present)	1.632 (1.000–2.662)	0.050	1.485 (0.907–2.432)	0.116
TNM stage (I~II vs. III~IV)	2.057 (1.237–3.421)	0.005	1.742 (1.025–2.960)	0.040*
Vessel carcinoma embolus (Absent vs. Present)	1.789 (1.048–3.055)	0.033	1.289 (0.732–2.272)	0.379
SCARNA16 expression (Low vs. High)	0.505 (0.308–0.829)	0.007	0.578 (0.345–0.969)	0.038*

*p<0.05. AFP, α-fetoprotein; ALT, alanine aminotransferase; CI, concordance index; HCC, hepatocellular carcinoma; HR, hazard ratio; TBil, total bilirubin; TFS, tumor-free survival; TNM, tumor-node-metastasis.

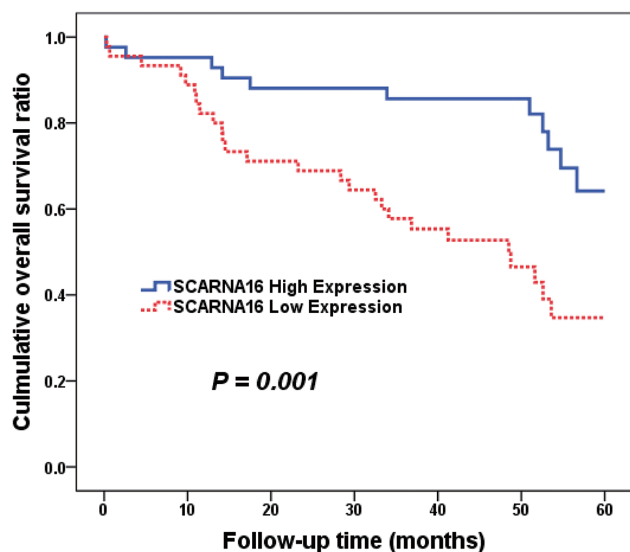

Fig. 4. Kaplan-Meier analysis in OS of HCC patients with low and high SCARNA16 expression. In the postoperative 5 years, HCC patients with low SCARNA16 expression tended to have shorter long-term survival time (*p*=0.001). OS, overall survival; HCC, hepatocellular carcinoma.

ent races, regions, liver disease background and medical technology levels, multiple medical centers should be united to perform the following research. More female and non-HBV-related HCC patients should also be enrolled into the research in the future. Besides, liver tissues collected from healthy people would help to determine the true baseline expression of SCARNA16 and verify its related carcinogenic mechanisms. Even so, these limitations did not compromise the integrity and scientificity of the present research.

In conclusion, the scaRNA SCARNA16 showed decreased expression pattern in both HCC cell lines and clinical specimens. Besides, we found significant association between low SCARNA16 level and several malignant characteristics, including large tumor size, vessel carcinoma embolus, capsular invasion, and severe TNM stages of HCC patients. During postoperative follow-up, HCC patients with low SCARNA16 expression generally manifested higher tumor recurrence rate and shorter long-term survival time. These findings suggested that SCARNA16 possesses potency as a novel biomarker for HCC.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceived of and designed the study (WW, SY, SZ), conducted the study (SZ, YD, ZS, YG, YC), contributed to the acquisition of data (QX, LZ, HX, YB), analyzed the data (ZS, SZ, CX, YL), interpreted the data (WW, ZS, XH, HD), and reviewed and edited the manuscript (WW, SY, SZ, YD, YG). All authors read and approved the manuscript.

Table 3. Univariate and multivariate analyses of OS in HCC patients

Clinicopathological parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age (<60 vs. ≥60 years)	0.864 (0.448–1.667)	0.663		
Sex (Female vs. Male)	0.885 (0.417–1.876)	0.750		
Hepatitis B (Negative vs. Positive)	1.059 (0.465–2.412)	0.892		
AFP (<200 vs. ≥200 ng/mL)	1.369 (0.710–2.641)	0.349		
Cirrhosis (Absent vs. Present)	1.219 (0.627–2.370)	0.559		
ALT (≤40 vs. >40 U/L)	0.780 (0.368–1.656)	0.518		
Serum TBil (≤17 vs. >17 μmol/L)	0.611 (0.268–1.392)	0.241		
Serum albumin (≥35 vs. <35 g/L)	1.539 (0.641–3.695)	0.335		
Tumor diameter (<5 vs. ≥5cm)	1.789 (0.817–3.917)	0.146		
Microvascular invasion (Absent vs. Present)	0.954 (0.293–3.110)	0.938		
Differentiation (High/moderate vs. Low)	0.691 (0.345–1.384)	0.297		
Vessel carcinoma embolus (Absent vs. Present)	0.954 (0.293–3.110)	0.938		
Capsular invasion (Absent vs. Present)	1.900 (0.994–3.629)	0.052	1.472 (0.755–2.871)	0.257
TNM stage (I~II vs. III~IV)	2.906 (1.520–5.556)	0.001	1.696 (0.799–3.596)	0.169
Multiple lesions (Absent vs. Present)	2.866 (1.449–5.669)	0.002	2.450 (1.221–4.915)	0.012*
SCARNA16 expression (Low vs. High)	0.329 (0.162–0.669)	0.002	0.366 (0.178–0.752)	0.006*

*$p<0.05$. AFP, α-fetoprotein; ALT, alanine aminotransferase; CI, concordance index; HCC, hepatocellular carcinoma; HR, hazard ratio; OS, overall survival; TBil, total bilirubin; TNM, tumor-node-metastasis.

Data sharing statement

The datasets used in support of the findings of this study are available from the corresponding author at wam@zju.edu.cn upon request.

References

[1] Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, *et al*. Hepatocellular carcinoma. Nat Rev Dis Primers 2016;2:16018. doi:10.1038/nrdp.2016.18.
[2] Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70(1):151–171. doi:10.1016/j.jhep.2018.09.014.
[3] Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019;16(10):589–604. doi:10.1038/s41575-019-0186-y.
[4] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70(1):7–30. doi:10.3322/caac.21590.
[5] Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev 2016;96(4):1297–1325. doi:10.1152/physrev.00041.2015.
[6] Slack FJ, Chinnaiyan AM. The role of non-coding RNAs in oncology. Cell 2019;179(5):1033–1055. doi:10.1016/j.cell.2019.10.017.
[7] Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. Nat Rev Cancer 2018;18(1):5–18. doi:10.1038/nrc.2017.99.
[8] Bohnsack MT, Sloan KE. Modifications in small nuclear RNAs and their roles in spliceosome assembly and function. Biol Chem 2018;399(11):1265–1276. doi:10.1515/hsz-2018-0205.
[9] Zhang PF, Wu J, Wu Y, Huang W, Liu M, Dong ZR, *et al*. The lncRNA SCARNA2 mediates colorectal cancer chemoresistance through a conserved microRNA-342-3p target sequence. J Cell Physiol 2019;234(7):10157–10165. doi:10.1002/jcp.27684.
[10] Ronchetti D, Todoerti K, Tuana G, Agnelli L, Mosca L, Lionetti M, *et al*. The expression pattern of small nucleolar and small Cajal body-specific RNAs characterizes distinct molecular subtypes of multiple myeloma. Blood Cancer J 2012;2(11):e96. doi:10.1038/bcj.2012.41.
[11] Ronchetti D, Mosca L, Cutrona G, Tuana G, Gentile M, Fabris S, *et al*. Small nucleolar RNAs as new biomarkers in chronic lymphocytic leukemia. BMC Med Genomics 2013;6:27. doi:10.1186/1755-8794-6-27.
[12] Mak LY, Cruz-Ramón V, Chinchilla-López P, Torres HA, LoConte NK, Rice JP, *et al*. Global epidemiology, prevention, and management of hepatocellular carcinoma. Am Soc Clin Oncol Educ Book 2018;38:262–279. doi:10.1200/EDBK_200939.
[13] Couri T, Pillai A. Goals and targets for personalized therapy for HCC. Hepatol Int 2019;13(2):125–137. doi:10.1007/s12072-018-9919-1.
[14] Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology 2016;150(4):835–853. doi:10.1053/j.gastro.2015.12.041.
[15] Kulik L, El-Serag HB. Epidemiology and management of hepatocellular carcinoma. Gastroenterology 2019;156(2):477–491.e1. doi:10.1053/j.gastro.2018.08.065.
[16] Chan SL, Wong AM, Lee K, Wong N, Chan AK. Personalized therapy for hepatocellular carcinoma: where are we now? Cancer Treat Rev 2016;45:77–86. doi:10.1016/j.ctrv.2016.02.008.
[17] Lee YT, Tan YJ, Oon CE. Molecular targeted therapy: Treating cancer with specificity. Eur J Pharmacol 2018;834:188–196. doi:10.1016/j.ejphar.2018.07.034.
[18] Klingenberg M, Matsuda A, Diederichs S, Patel T. Non-coding RNA in hepatocellular carcinoma: mechanisms, biomarkers and therapeutic targets. J Hepatol 2017;67(3):603–618. doi:10.1016/j.jhep.2017.04.009.
[19] Wang Y, Chen F, Zhao M, Yang Z, Li J, Zhang S, *et al*. The long noncoding RNA HULC promotes liver cancer by increasing the expression of the HMGA2 oncogene via sequestration of the microRNA-186. J Biol Chem 2017;292(37):15395–15407. doi:10.1074/jbc.M117.783738.
[20] Xu H, Wang C, Song H, Xu Y, Ji G. RNA-Seq profiling of circular RNAs in human colorectal cancer liver metastasis and the potential biomarkers. Mol Cancer 2019;18(1):8. doi:10.1186/s12943-018-0932-8.
[21] Fakan S, Leser G, Martin TE. Ultrastructural distribution of nuclear ribonucleoproteins as visualized by immunocytochemistry on thin sections. J Cell Biol 1984;98(1):358–363. doi:10.1083/jcb.98.1.358.
[22] Meier UT. RNA modification in Cajal bodies. RNA Biol 2017;14(6):693–700. doi:10.1080/15476286.2016.1249091.
[23] Nagasawa C, Ogren A, Kibiryeva N, Marshall J, O'Brien JE, Kenmochi N, *et al*. The role of scaRNAs in adjusting alternative mRNA splicing in heart development. J Cardiovasc Dev Dis 2018;5(2):26. doi:10.3390/jcdd5020026.
[24] Mahmoudi S, Henriksson S, Weibrecht I, Smith S, Söderberg O, Strömblad S, *et al*. WRAP53 is essential for Cajal body formation and for targeting the survival of motor neuron complex to Cajal bodies. PLoS Biol 2010;8(11):e1000521. doi:10.1371/journal.pbio.1000521.
[25] Ronchetti D, Todoerti K, Tuana G, Agnelli L, Mosca L, Lionetti M, *et al*. The expression pattern of small nucleolar and small Cajal body-specific RNAs characterizes distinct molecular subtypes of multiple myeloma. Blood Cancer J 2012;2:e96. doi:10.1038/bcj.2012.41.
[26] Teittinen KJ, Laiho A, Uusimäki A, Pursiheimo JP, Gyenesei A, Lohi O. Expression of small nucleolar RNAs in leukemic cells. Cell Oncol (Dordr) 2013;36:55–63. doi:10.1007/s13402-012-0113-5.
[27] Ronchetti D, Mosca L, Cutrona G, Tuana G, Gentile M, Fabris S, *et al*. Small nucleolar RNAs as new biomarkers in chronic lymphocytic leukemia. BMC Med Genomics 2013;6:27. doi:10.1186/1755-8794-6-27.
[28] Chu L, Su MY, Maggi LB Jr, Lu L, Mullins C, Crosby S, *et al*. Multiple myeloma-associated chromosomal translocation activates orphan snoRNA ACA11 to suppress oxidative stress. J Clin Invest 2012;122(8):2793–2806. doi:10.1172/JCI63051.
[29] Cao T, Rajasingh S, Samanta S, Dawn B, Bittel DC, Rajasingh J. Biology and clinical relevance of noncoding sno/scaRNAs. Trends Cardiovasc Med 2018;28(2):81–90. doi:10.1016/j.tcm.2017.08.002.
[30] Xie DY, Ren ZG, Zhou J, Fan J, Gao Q. 2019 Chinese clinical guidelines for the management of hepatocellular carcinoma: updates and insights. Hepatobiliary Surg Nutr 2020;9(4):452–463. doi:10.21037/hbsn-20-480.