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Expression of Rho Guanine Nucleotide Exchange Factor 39 (ARHGEF39) and Its Prognostic Significance in Hepatocellular Carcinoma

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Previous studies have reported that ARHGEF39 might be frequently upregulated in different cancer types and relevant to cancer progression. However, the expression pattern and clinicopathological features of ARHGEF39 in patients with hepatocellular carcinoma (HCC) needs further exploration.

Material/Methods: ARHGEF39 expression level of HCC in The Cancer Genome Atlas (TCGA) dataset was analyzed. Quantitative real-time polymerase chain reaction and immunohistochemistry were employed to determine ARHGEF39 mRNA and protein levels in our own study collected HCC tissues and matched non-cancerous tissues. Moreover, the association of ARHGEF39 expression with the clinicopathological factors and prognosis of HCC were investigated.

Results: The level of ARHGEF39 in HCC tissues was significantly higher than that in adjacent normal tissues ($P<0.05$) from TCGA database. High level of ARHGEF39 was a significant prognostic factor of poor overall survival (OS) (TCGA, $P=0.006$). Consistently, the expression levels of ARHGEF39 mRNA and protein in HCC specimens were significantly higher than those in adjacent liver specimens ($P<0.05$) from our cohort. Further analysis revealed that high ARHGEF39 level was significantly associated with poor OS ($P<0.001$) and short disease-free survival (DFS) ($P<0.001$). Cox multivariate analysis indicated that ARHGEF39 was an independent, unfavorable prognostic factor ($P=0.000$) of OS and DFS.

Conclusions: ARHGEF39 might act as an oncogene in the progression of HCC and might serve as a promising potential prognostic indicator and therapeutic target for HCC.

MeSH Keywords: Carcinoma, Hepatocellular • Prognosis • rho GTP-Binding Proteins

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Background

Hepatocellular carcinoma (HCC) is a major pathological type of primary liver cancer. The death it causes has become the fourth leading cause of cancer death in the world and remains second in the mortality rate of malignant tumors in China [1,2]. Although the early diagnosis, surgical treatment, and comprehensive therapy of HCC have advanced greatly in recent years, the prognosis for HCC remains poor because of its high rates of local recurrence and distant metastasis [3].Therefore, it is of critical importance to discover and validate novel diagnostic and prognostic biomarkers of HCC.

The Rho family of small GTPases plays crucial roles in actin cytoskeleton regulation, cell polarity, cell migration, and cell proliferation [4–7]. Previous studies have shown that the abnormal regulation of GTP enzymes in the RHO family might be related to the occurrence and migration of tumors and is regulated by the Dbl family of guanine nucleotide exchange factors (GEFs) [8,9]. Several Dbl-family GEFs have been found to be implicated in development and progression of tumors. For example, elevated of Tiam1 promotes lung cancer metastasis and is correlated with poor outcome via epithelial-mesenchymal transition [10]. Tiam2 enhances cell invasion and motility in liver cancer cells via Sp1-mediated transcriptional activation [11].

ARHGEF39, also known as C9orf100, is a member of the human Dbl family of guanine nucleotide exchange factors (RhoGEFs) which are important activators of Rho family small GTPases [12]. The overexpression of ARHGEF39 has also been identified in various human malignancies, including non-small cell lung cancer [13], gastric cancer [14],and hepatocellular carcinoma [15]. Together these data suggest that ARHGEF39 might be frequently upregulated in different cancer type and associated with cancer progression. Previously, it was reported that the mRNA level of ARHGEF39 in HCC samples was significantly upregulated compared to that of adjacent non-tumor tissues. Increased expression of ARHGEF39 mRNA was positively correlated with the number of nodules and with serum alpha-fetoprotein (AFP) levels [15]. However, we still have little understanding of the expression and prognostic relevance of ARHGEF39 protein in HCC. The objective of this research was to thoroughly analyze ARHGEF39 protein expression patterns and its potential role as diagnostic and prognostic biomarkers in HCC.

Material and Methods

Patients and clinical specimens

We included 135 archival paraffin-embedded HCC tissues and their paracancerous tissues from the pathological specimen database of our hospital between February 2009 and December 2013. The details of the patient are shown in Table 1. The median age of the 135 patients was 52.9 years old (ranging from 25 to 85 years old). Among the 135 patients, 50 patients were 50 years old or younger, and 85 patients were older than 50 years of age. A total of 45 tumors were classified as poorly differentiated, while 90 were classified as moderately or highly differentiated. Inclusion criteria were: patients histologically confirmed HCC by experienced pathologists; all patients who had received no radiotherapy or chemotherapy before hepatectomy; and according to the TNM classification of the International Union against Cancer (UICC) in 2003, all the patients were classified into I, II, III, or IV stage. Whereas exclusion criteria were patients who have received any prior antitumor therapies. In addition, 19 pairs of fresh samples of human HCC and corresponding paracancerous tissues (at least 2 cm away from the tumor edge) of HCC were obtained for quantitative real-time polymerase chain reaction (qRT-PCR) analysis from the same hospital. Samples were stored at -80°C until use. The follow-up ended on June 30, 2018, or when patients died. The median follow-up was 46.1 months (ranging 1.0 to 112 months). Ethical approval was given by the medical ethics committee of our institution and all participants.

Data mining in The Cancer Genome Atlas (TCGA) for HCC

Datasets of HCC were downloaded from the Cancer Genome Atlas (TCGA) database to evaluate the mRNA level of ARHGEF39 in HCC tissues. We firstly obtained the RNAseq data of 374 HCC tissues and 50 paracancerous tissues together with the clinical data. Secondly, we detected the expression of ARHGEF39 from mRNA profiles and then, we forecasted the mRNA expression of ARHGEF39 by mining TCGA database and assessed their prognostic value by using the Kaplan-Meier survival curves in HCC patients

Immunochemical staining and evaluation

According to the manufacturer's protocol, immunohistochemistry was performed with ARHGEF39 primary antibody (HPA061299, Sigma) at a dilution of 1: 100. The results were determined using a blind method, and each slice was counted by 2 pathologists. The immunoreactive score (IRS) was calculated according to the staining intensity (SI) multiplied by the percentage of staining positive cells (PP). The detailed score standards were as follows: SI (ranging from 0–3 scores): 0 was negative, 1 was weak, 2 was moderate and 3 was strong; the percent positivity was scored as 0 (0, negative), 1 (1% to 25%,), 2 (26% to 50%), 3 (51% to 75%), and 4 (76% to 100%). The IRS score ranged from 0 to 12. High expression of ARHGEF39 was defined as IRS >4, and low expression of ARHGEF39 was defined as IRS ≤4.

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Table 1. Analysis of the relationship between expression of ARHGEF39 and various clinicopathological parameters.

Variables	Cases (N)	ARHGEF39 expression		χ^2	P value
		High	Low		
Age at surgery (years)					
≤50	50	25	25	1.280	0.285
>50	85	51	34		
Gender					
Male	114	68	46	3.348	0.093
Female	21	8	13		
sAFP (ng/mL)					
≤20	49	19	30	9.597	**0.002**
>20	86	57	29		
HbsAg					
Positive	107	60	47	0.010	1.000
Negative	28	16	12		
Tumor size (cm)					
>5	79	45	34	0.034	0.862
≤5	56	31	25		
Tumor nodules					
Multiple	29	21	8	3.900	0.058
Single	106	55	51		
Vascular invasion*					
Present	34	23	11	2.380	0.162
Absent	101	53	48		
Tumor capsule					
Complete	97	52	45	1.012	0.341
None	38	24	14		
TNM stage					
I–II	92	46	46	4.654	**0.040**
III–IV	43	30	13		
Differentiation					
Poor	45	28	17	0.963	0.361
Moderate/high	90	48	42		
Cirrhosis					
Present	106	61	45	0.314	0.314
Absent	29	15	14		
BCLC stage					
A–B	116	63	53	1.321	0.321
C–D	19	13	6		
Child-Pugh					
A	127	70	57	1.209	0.465
B	8	6	2		

HBsAg – hepatitis B surface antigen; sAFP – serum alpha-fetoprotein; TNM – tumor-node-metastasis; BCLC – Barcelona clinic liver cancer. * Macroscopic or microscopic cancer thrombus of HCC.

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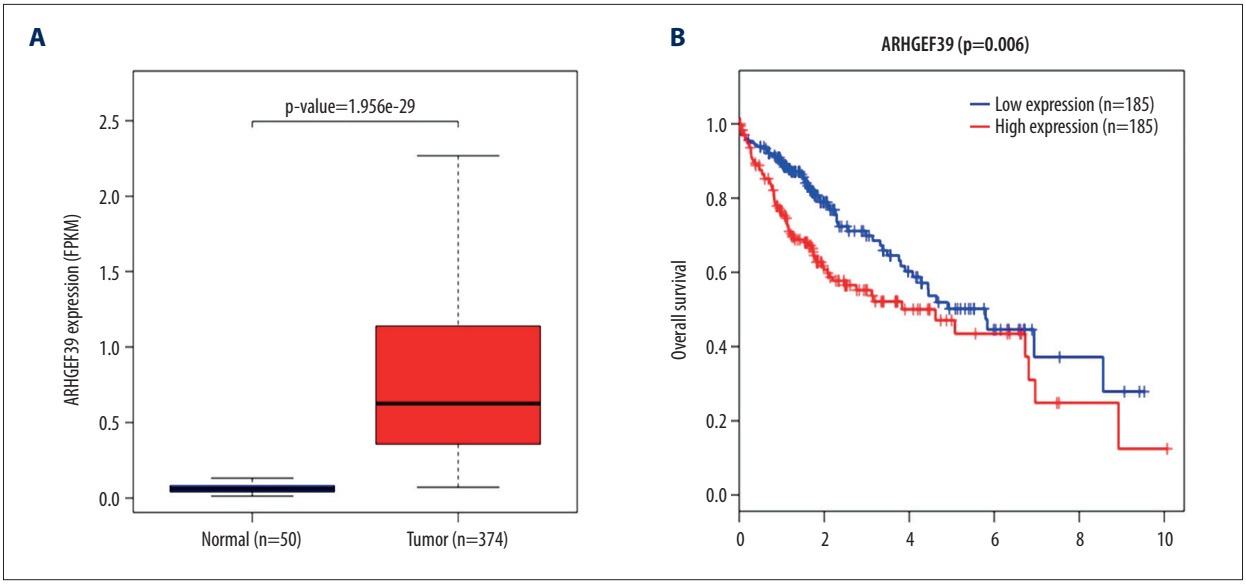

Figure 1. Relationship between ARHGEF39 mRNA and the prognosis of hepatocellular carcinoma (HCC) patients predicted by The Cancer Genome Atlas (TCGA) database. TCGA database mining analysis of (**A**) ARHGEF39 mRNA levels grouped by HCC and normal liver and (**B**) relationship between ARHGEF39mRNA and the prognosis of HCC patients.

The qRT-PCR assays

Total RNA was extracted from the 19 pairs of fresh-frozen cancerous and paracancerous tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, United States), quantification and concentration of total RNA was determined using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA) and Invitrogen Qubit 3.0 Spectrophotometer (Thermo Fisher Scientific, USA). Then RNA was reverse transcribed into complementary DNA (cDNA) using a PrimeScript RT reagent kit (Takara Bio Inc., Japan). The qRT- PCR assays were conducted using the 7900HT Fast Real-Time PCR System (ABI, USA) and SYBR-Green I reagent (Sigma, USA). The ARHGEF39 mRNA level in each sample was normalized to human GAPDH. The primer sets used were:
ARHGEF39, forward: 5'-GGATCCTGAAAGCCAAGGGG-3',
reverse: 5'-TCCAGGTAGGGAAGCAGCTC-3';
GAPDH, forward: 5'-GGAGTCCACTGGCGTCTTCA-3',
reverse: 5'-GCAGAGGand GGGCAGAGATGAT-3'.

Relative mRNA expression levels were analyzed by using the $2^{-\Delta\Delta Ct}$ method. Experiments were performed at least 3 times independently.

Statistical analysis

We used SPSS 19.0 software to perform the statistical analysis. The chi-square test was used to determine the relationship between ARHGEF39 expression and the clinicopathological factors of HCC patients. Kaplan-Meier and Log-rank test were employed to identify the variables related to disease-free survival (DFS) and overall survival (OS) in HCC patients. The statistically significant parameters in univariate analysis were selected for Cox multivariate analysis to determine its prognostic value. $P<0.05$ indicates that the difference was statistically significant.

Results

High frequency expression of ARHGEF39 mRNA and protein in HCC

Firstly, the mRNA expression profiles of ARHGEF39 in HCC and normal live tissues was shown by the bar graph in Figure 1A ($P<0.001$). Genome data of 374 independent HCC patients downloaded by TCGA (including 50 pairs of cancer and adjacent tissues) showed that ARHGEF39 was significantly upregulated in HCC patients compared with adjacent tissues. Then, in order to authenticate the veracity of the expression of ARHGEF39 in HCC, we used qRT-PCR and immunohistochemistry techniques to assess ARHGEF39 expression in cancer and noncancerous tissues (Figures 2, 3), at the transcriptional level, The median ARHGEF39 mRNA level was remarkably increased in HCC tissues compared to peritumor liver ($P<0.05$), as depicted in Figure 2. The results of immunohistochemical staining (Figure 3) showed that ARHGEF39 mainly appeared in the cell membrane and cytoplasm of HCC cancer cells. High ARHGEF39 expression levels were detected in 76 of the 135 (56.3%) HCC cancer tissues (Table 2), compared with 17.0% (23 out of 135) in corresponding adjacent tissues. In order to confirm the functionality of ARHGEF39 in HCC, analysis of the

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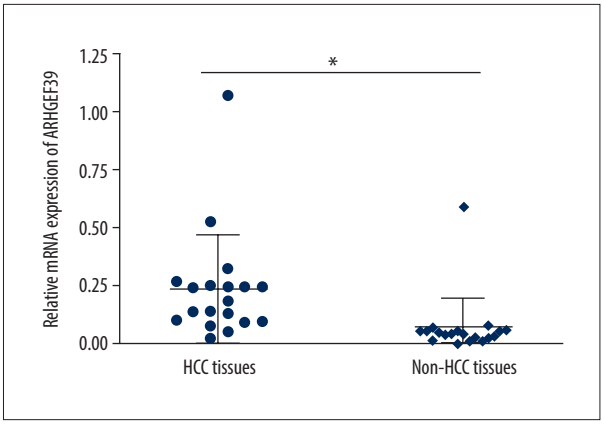

Figure 2. Quantitative real-time polymerase chain reaction analysis of ARHGEF39 mRNA expression in 19 pairs of hepatocellular carcinoma tissues and noncancerous liver tissues (* $P<0.05$).

Table 2. High ARHGEF39 expression levels were detected in 135 cases of HCC cancer tissue compared with the corresponding adjacent tissues.

	ARHGEF39 expression level	
	High (n)	Low (n)
HCC cancer tissues	76	59
Paracancerous tissues	23	112
χ^2	44.801	
P	0.000	

HCC – hepatocellular carcinoma.

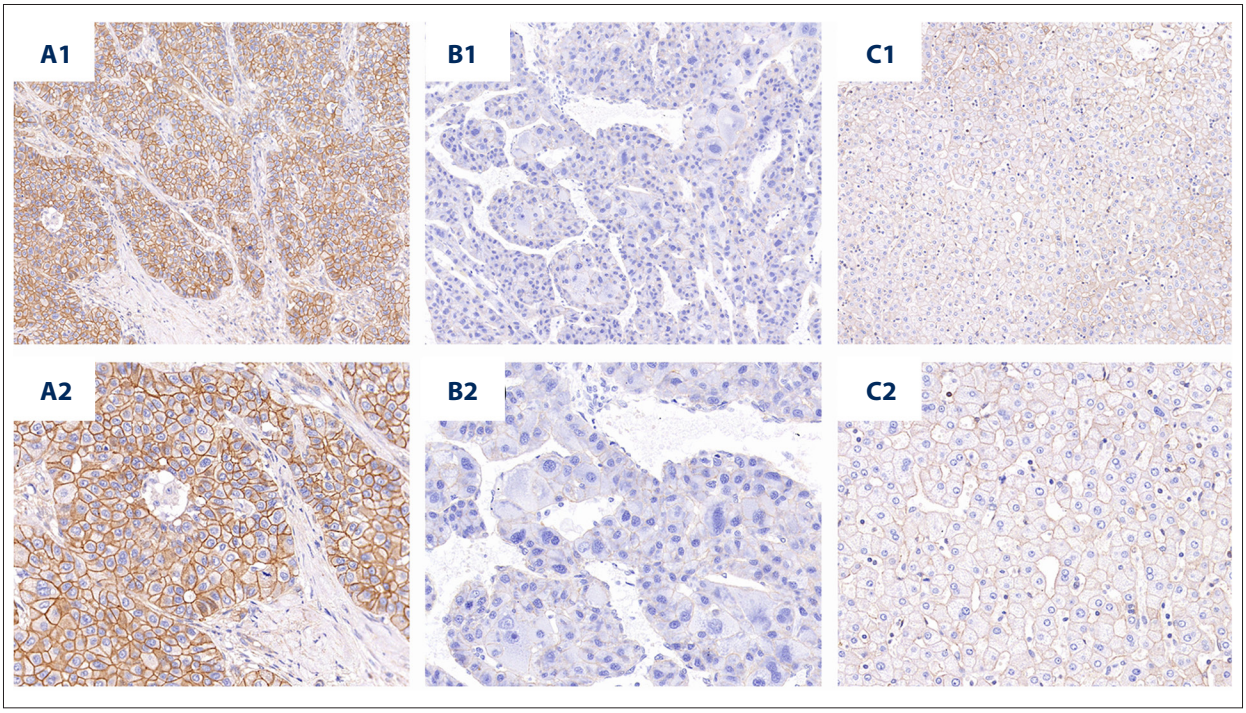

Figure 3. Representative immunohistochemical staining of ARHGEF39 in 135 cases of hepatocellular carcinoma (HCC) and matched adjacent normal tissues (**A1, A2**). High expression of ARHGEF39 in HCC tissues (**B1, B2**). Low expression of ARHGEF39 in HCC tissues (**C1, C2**). Low expression of ARHGEF39 in paired adjacent normal tissue (**A1, B1, C1** with original magnification of 200×; and **A2, B2, C2** with original magnification of 400×).

relationship between expression of ARHGEF39 and various clinicopathological parameters is shown in Table 1. Overexpression of ARHGEF39 in 135 HCCs was associated with an elevated AFP level (P=0.002, χ^2=9.597) and higher TNM stage (P=0.040, χ^2=4.654). The high ARHGEF39 expression group had significantly increased expression of AFP (>20), with 57 out of 76 or 75.0% versus 29 out of 59 or 49.2%) compared with the low ARHGEF39 expression group (Table 1, Figure 4A). In addition, the high ARHGEF39 expression group also had a significantly higher ratio of stages III/IV patients (30 out of 7, 39.5%) than low ARHGEF39 expression group (13 out of 59, 22.0%; P=0.040; Table 1, Figure 4B). In addition, there was no significant association between ARHGEF39 expression and age, gender, HbsAg, tumor size, tumor nodules, vascular invasion, tumor capsule, histological differentiation, cirrhosis, BCLC stage, or Child-Pugh score.

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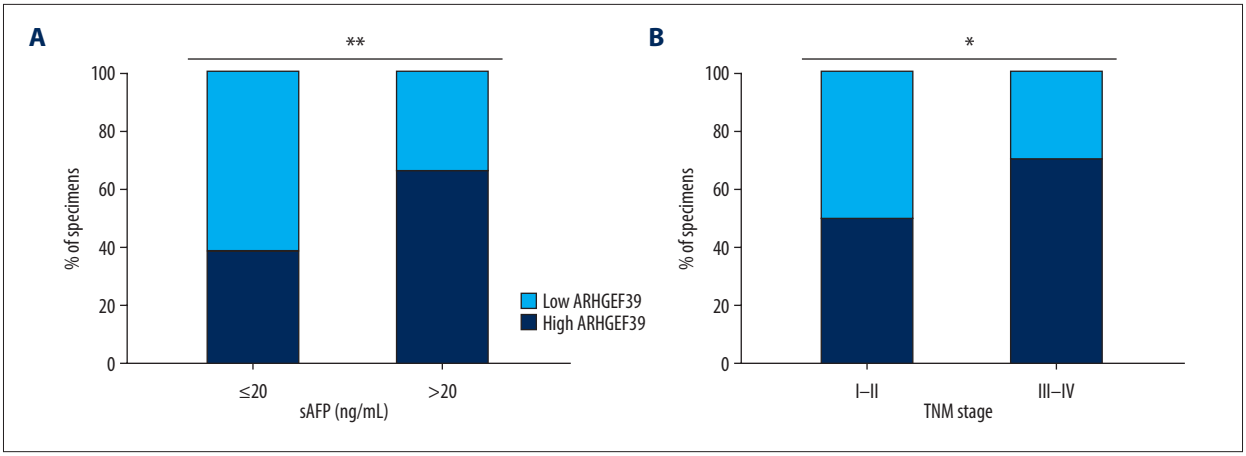

Figure 4. Correlation between the ARHGEF39 level of expression and (**A**) serum alpha-fetoprotein (AFP) level (** *P*<0.01) and (**B**) TNM stage (* *P*<0.05).

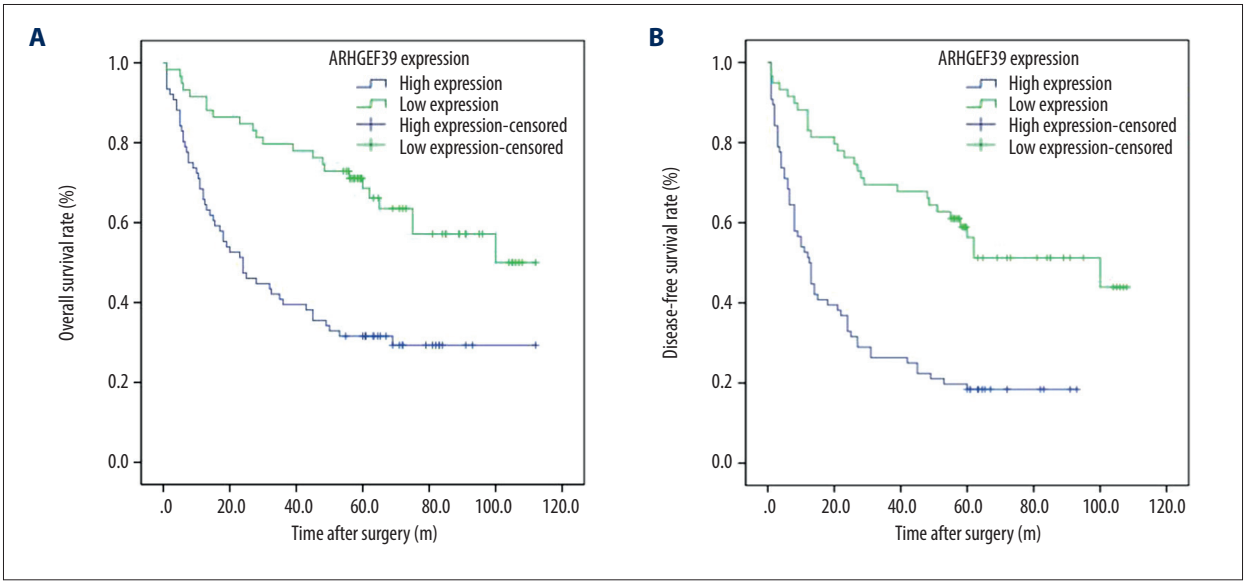

Figure 5. Kaplan-Meier estimates was performed for overall survival (OS) and disease-free survival (DFS) (**A**) OS curve of hepatocellular carcinoma hepatocellular carcinoma (HCC) patients associated with ARHGEF39 expression; (**B**) DFS curve of HCC patients associated with ARHGEF39 expression.

High expression of ARHGEF39 might rightly be ranked as one of the risk factors affecting the prognosis of HCC

The prognostic performance of high ARHGEF39 expression in HCC patients from the TCGA cohorts was evaluated by Kaplan-Meier risk estimates. Intriguingly, there was a positive correlation between the poorer OS rate and the higher expression of ARHGEF39 in patients with HCC (TCGA, *P*=0.006) (Figure 1B). Then, these findings were clearly verified by our own survival analysis data. As shown in Figure 5, higher expression of ARHGEF39 was significantly associated with worse OS and poorer DFS in HCC patients.

Independent prognostic value of ARHGEF39 expression in HCC patients after hepatectomy

The univariate analysis indicated that ARHGEF39 expression, vascular invasion, BCLC stage, TNM stage, and Child-Pugh score were directly correlated with both OS and DFS. Furthermore, by Cox multivariate survival analysis, we found that high ARHGEF39 expression, TNM stage, and Child-Pugh score were confirmed as independent prognostic indicators influencing the prognosis of HCC patients (Tables 3, 4).

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Table 3. Univariate analysis of prognostic factors of OS and DFS in patients with HCC.

Variables	OS			DFS		
	Mean	95% CI	*P*	Mean	95% CI	*P*
ARHGEF39						
High	46.043	35.906–56.181	**0.000**	28.355	20.869–35.842	**0.000**
Low	79.673	69.121–90.225		69.056	58.152–79.960	
Age						
≤50	57.77	44.216–71.316	0.634	44.908	31.737–58.079	0.620
>50	61.73	52.127–71.340		48.210	39.398–57.021	
Gender						
Male	57.865	49.252–66.478	0.101	44.734	36.640–52.828	0.091
Female	75.252	58.688–91.815		63.754	44.931–82.577	
sAFP (ng/mL)						
≤20	66.313	53.674–78.952	0.269	50.333	38.809–61.857	0.518
>20	56.444	46.519–66.370		45.075	35.537–54.613	
Tumor size (cm)						
>5	46.369	36.702–56.037	**0.000**	38.540	29.375–47.706	**0.009**
≤5	81.418	70.350–92.487		60.049	48.280–71.819	
Tumor nodules						
Multiple	44.017	28.522–59.513	**0.037**	35.569	20.639–50.499	0.090
Single	64.653	55.891–73.416		50.706	42.192–59.221	
Vascular invasion						
Present	41.906	28.029–55.782	**0.004**	29.176	16.679–41.672	**0.001**
Absent	67.168	58.208–76.128		54.427	45.616–63.239	
Tumor capsule						
Complete	65.869	56.541–75.197	**0.048**	52.233	43.033–61.434	**0.062**
None	47.441	34.018–60.865		37.004	24.691–49.318	
TNM stage						
I–II	77.266	68.415–86.117	**0.000**	60.097	50.856–69.339	**0.000**
III–IV	26.363	16.824–35.902		21.907	12.793–31.021	
Differentiation						
Poor	50.014	35.817–64.211	0.052	33.598	21.736–45.459	0.010
Moderate/high	65.887	56.658–75.116		54.109	44.947–63.271	
Cirrhosis						
Present	62.907	54.008–71.806	0.285	48.433	39.957–56.910	0.735
Absent	51.764	35.960–67.568		45.636	29.323–61.950	
BCLC stage						
A–B	65.472	57.050–73.895	**0.001**	51.531	43.300–59.762	**0.006**
C–D	32.921	15.673–50.169		25.289	9.821–40.758	
Child-Pugh						
A	63.510	55.457–71.562	**0.000**	49.757	41.952–57.561	**0.001**
B	16.625	0.000–34.835		16.000	0.000–34.237	

OS – overall survival; DFS – disease-free survival; HBsAg – hepatitis B surface antigen; AFP – alpha-fetoprotein; TNM – tumor-node-metastasis; BCLC – Barcelona clinic liver cancer; CI – confidence interval.

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Table 4. Multivariate Cox regression analysis of patients' OS and DFS.

Variable	OS			DFS		
	RR	95% CI	*P*	RR	95% CI	*P*
ARHGEF39 expression (low *vs.* high)	0.342	0.198–0.592	**0.000**	0.366	0.224–0.597	**0.000**
Tumor size (≤5 *vs.* >5)	0.436	0.246–0.773	**0.004**	0.705	0.435–1.141	0.155
Tumor nodule (single *vs.* multiple)	1.210	0.683–2.144	0.513	1.071	0.630–1.821	0.801
Tumor capsule (complete *vs.* none)	0.633	0.350–1.144	0.130	0.720	0.401–1.294	**0.002**
Vascular invasion (present *vs.* absent)	0.867	0.492–1.529	0.623	0.771	0.451–1.319	0.342
TNM stage (I/II *vs.* III/IV)	3.282	1.812–5.944	**0.000**	2.540	1.397–4.618	**0.002**
Differentiation (poor *vs.* moderate/high)	0.993	0.602–1.637	0.978	0.828	0.529–1.295	0.408
BCLC stage (A–B *vs.* C–D)	1.513	0.755–3.032	0.243	1.113	0.577–2.147	0.749
Child-Pugh classification (A *vs.* B)	6.352	2.734–14.758	**0.000**	3.522	1.585–7.823	**0.002**

OS – overall survival; DFS – disease-free survival; RR – relative risk; CI – confidence interval; TNM – tumor-node-metastasis; BCLC – Barcelona clinic liver cancer.

Discussion

In this study, patients' information and gene expression profiles in the TCGA database were initially studied to predict that ARHGEF39 mRNA expression in liver cancer tissues and were found to be significantly higher than that in corresponding peritumor tissues. Then, immunohistochemical staining was performed to validate the expression level of ARHGEF39 at the protein level. We found that ARHGEF39 protein was mainly located in cell membrane and cytoplasm, and its positive expression rate in HCC was clearly higher than that in pericancerous tissues. Our own experimental data verified the results of the bioinformatics assay. In addition, we also found that the high expression of ARHGEF39 in cancerous tissues revealed a positive correlation with the levels of serum AFP and might be closely correlated with enhanced TNM stage of HCC. Collectively, our data and these studies indicated that ARHGEF39 is not only a useful predictive marker of early emerging HCC in patients but also might play a key role in the progress of HCC.

Based on the results of our univariate analysis, we demonstrated that tumor size >5 cm, vascular invasion, stage III-IV of TNM, stage C-D of BCLC, and Child-Pugh B score were responsible for the lower DFS and OS. This result is consistent with the earlier report which states that tumor size is a highly significant prognostic variable for local recurrence of HCC. The 5-year recurrence rate for HCC <5 cm was significantly lower than those for HCC ≥5 cm, tumor size might be a significant prognostic factor for recurrence and overall survival [16,17]. Vascular invasion, whether macrovascular or microvascular invasion, is a marker of aggressive biological behavior of tumors and is currently one of the most relevant risk factors predictive of HCC recurrence [18,19]. HCC has a high tendency to invade the portal vein system and forms a portal vein cancer thrombus (PVTT), which is the most common form of macroscopic tumor thrombus [20,21]. PVTT is an important prognostic factor in patients with HCC and multivariate analyses have shown it to be a significant and independent prognostic factor that influences patient survival [22]. The formation of portal vein tumor thrombus seriously hinders the surgical treatment of patients with HCC. Even if the operation is carried out, it is easy to metastasis and recurrence in a short period of time, which affects the prognosis of the patients. The presence of microvascular invasion in HCC was assessed based on pathological reports from surgical specimens and was defined as tumor thrombus in the hepatic veins, portal venous system and/or lymphatic ducts that was visible only on microscopy [23,24]. Microvascular invasion is also considered an important predictor for postoperative recurrence and prognosis of HCC [25]. The Child-Pugh classification was often used to evaluate the preoperative liver function in patients with HCC. In our study, the OS observed was significantly different between patients with Child-Pugh A and Child-Pugh B (median OS was 63.5 months versus 16.6 months, $P<0.001$). This result was consistent with recent findings by Kong et al. (2017) who reported that Child-Pugh classification was a prognostic factor for OS, and the OS of patients in the class A group was significantly longer than that of the class B group (median OS was 31 months versus 18 months, $P<0.001$) [26]. The BCLC stage was originally developed for patients undergoing HCC resection and was more accurate than indocyanine green clearance test and Child-Pugh score in predicting survival after resection in patients with HCC [27]. The BCLC stage performed better than other multidimensional staging systems, even after stratification by curative or palliative treatment. This scoring system seems to be particularly useful for predicting individual HCC prognosis in clinical practice [28].

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The findings by the Cox multivariate survival analysis indicated that ARHGEF39 overexpression, stage III–IV of TNM and Child-Pugh score were independent prognostic indicators for survival of patients with HCC. This was in agreement with a previous study showing that advanced TNM stage was associated with unfavorable survival in HCC [29]. And the rates of 1-year, 3-year, and 5-year DFS and OS were significantly higher in TNM stage I group compared with that in TNM stage II–III group [29].

Overexpression of ARHGEF39 was shown to increased Akt activation but had no effect on ERK activation. Knocking down ARHGEF39 was shown to inhibit gastric cancer cells migration, invasion, and tumorigenic growth. Downregulation of ARHGEF39 expression using an RNA silencing approach in gastric cancer cells inhibited migration and invasion in gastric cancer cell lines, as well as suppressed *in vivo* tumor growth and metastasis in a nude mouse xenograft model [14]. Upregulated ARHGEF39 protein expression has also been seen in lung adenocarcinoma cells trains and tissues compared to normal bronchial epithelial cells, and siRNA-directed inhibition of ARHGEF39 inhibited lung cancer cells growth and invasion and was involved in the regulation of P38-ATF2 signaling pathway [13]. Furthermore, it was also reported that ARHGEF39 overexpression promoted the cell invasion and migration abilities of HCC cancer cells, whereas knockdown of this target gene by RNA interference inhibited these processes of human HCC MHCC-LM6 cells [15].

Invasion and metastasis are the most significant and intrinsic biological characteristics of cancers, the progress of neoplasm metastasis is associated with poor survival. Frequent metastasis causes early postoperative recurrence and poor clinical outcome in HCC patients. In our study, Kaplan-Meier analysis showed that DFS and OS of ARHGEF39 upregulated group were significantly shorter. In addition, multivariate Cox analysis demonstrated that overexpression of ARHGEF39 was an independent predictor of HCC outcome. We conjecture that the increased expression of ARHGEF39 in HCC may improve tumor invasion and metastasis by increasing the migration capacity of tumor cells, resulting in a worse prognosis in patient with HCC.

However, there were several limitations to our study that must be considered. First, the patients involved in this study were all from the Chinese population and most of them were hepatitis B patients. Other factors, including lifestyle patterns (cigarette smoking, habitual alcohol drinking), aflatoxin exposure, and hepatitis C virus (HCV) superinfection might also influence survival in patients with hepatitis B virus (HBV)-related liver failure [30]. In addition, this was a retrospective, single center-institutional analysis, limited sample size, as well as bias caused by single-center analysis. Large multicenter trials are needed to investigate in the future. Finally, we have not explored the upstream and downstream of signal transduction pathways in this study, which will be clarified in future research.

Conclusions

We found that overexpression of ARHGEF39 was confirmed in approximately 60% the HCC specimens analyzed. ARHGEF39 expression was in HCC as compared to their paired paracancerous samples. Upregulated ARHGEF39 was closely related with several clinicopathological features of HCC which associated with occurrence and progression of HCC. Importantly, univariate and multivariate survival analyses identified the elevated ARHGEF39 expression in HCC as independent risk factors of both shorter DFS and OS. Future studies should take these risk factors into account.

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