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Down-regulation of miR-655-3p predicts worse clinical outcome in patients suffering from hepatocellular carcinoma

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Abstract. – OBJECTIVE: MiR-655-3p has been reported to play important roles in tumor initiation, development, and metastasis in several cancers. This study aimed to assess the potential role of miR-655-3p in the pathogenesis of hepatocellular carcinoma (HCC).

PATIENTS AND METHODS: The expression levels of miR-655-3p in HCC tissues were detected by qPCR. The relationship between clinicopathologic characteristics and miR-655-3p was analyzed by chi-square test. Kaplan-Meier curves and multivariate Cox proportional models were used to study the impact on clinical outcome.

RESULTS: miR-655-3p was significantly down-regulated in HCC tissues compared to normal adjacent liver tissues ($p < 0.01$). Low miR-655-3p expression was observed to be closely correlated with positive microvascular invasion, advanced tumor stage and lymph node metastasis ($p < 0.05$, respectively). The results of Kaplan–Meier analysis showed that patients with high miR-655-3p expression lived shorter than those with low miR-655-3p expression (Log-rank test, $p = 0.0002$). Multivariate analysis revealed that miR-655-3p was an independent risk factor for HCC (HR=1.533, 95% CI: 0.988-3.891; $p = 0.002$).

CONCLUSIONS: Our data showed that low expression of miR-655-3p was associated with significant characteristics of patients with HCC, and it could function as a potential unfavorable prognostic biomarker.

Key Words:
MiR-655-3p, Hepatocellular carcinoma, Prognosis.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third most common cause of cancer death globally[1]. Despite the recent advances in clinical and experimental oncology, the prognosis of HCC still remains poor[2,3]. Notably, most patients diagnosed with advanced-stage HCC die of recurrence and/or metastasis[4]. Thus, it is important to identify new markers to predict more accurately prognosis of an individual patient.

MicroRNAs (miRNAs) are single-stranded, small noncoding RNAs of 18-22 nucleotides in length, first discovered in the early 1990s in *Caenorhabditis elegans*[5]. It has been known to us that miRNAs regulate the expression of various genes at the post-transcriptional level by binding to the 3'-untranslated region (3'-UTR) of their target mRNAs[6,7]. Due to their widespread modulation on protein-coding genes, miRNAs have various physiological and pathological functions[8]. Numerous studies have demonstrated that miRNAs can function as oncogenes or tumor suppressors[9,10]. Currently, some studies revealed that changes in expression of miRNAs may be used as robust biomarkers for cancer risk, diagnosis, and prognosis. Zhang et al[11] showed that low miR-613 expression was associated with lower progression-free and overall survival. Chen et al[12] found that serum miR-182 and miR-331-3p were associated with postoperative survival of HCC patients, and they further identified them to be independent prognostic factors for patients with HCC. A previous paper[13] reported that miR-655-3p was significantly down-regulated in HCC tissues and HCC cell lines, and overexpression of miR-655-3p suppressed cell proliferation and migration in HCC by directly targeting ADAM10. However, the clinical significance of miR-655-3p was never reported in HCC.

In the present work, we performed RT-PCR to determine the expression levels of miR-655-3p. Subsequently, we analyzed the association between the miR-655-3p expression and various clinicopathological factors of HCC patients. Fi-

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nally, we further studied the correlation between miR-655-3p expression and prognostic value.

Patients and Methods

Patients and Tissue Samples

HCC tissue slice samples were obtained from 188 patients diagnosed with HCC who had undergone a curative hepatectomy in the Chinese PLA General Hospital. After resection, frozen HCC tissues were collected immediately after resection. All the diagnosis was pathologically confirmed. None of the patients had undergone preoperative intervention therapy or chemotherapy. The clinicopathological information of the patients is summarized in Table I. Written informed consent for the biological studies was obtained from each patient involved in the study. The study was approved by the Ethical Review Committee of the Institute. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration.

RNA Isolation and Quantitative Real-Time PCR

Total RNA from tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) for mRNA analyses. cDNA was synthesized with the ReverTra Ace®qPCR RT Kit (FSQ-101; Toyobo, Osaka, Japan). Real-time PCR was used to determine the expression of miR-655-3p in HCC tissues. Quantitative real-time RT-PCR was performed by using the SYBR green reagent with an ABI Prism 7000HT sequence detection system. Primers for miR-655-3p and the internal control GAPDH gene were purchased from Ambion (Applied Biosystems, Foster City, CA, USA). Relative quantification of miR-655-3p expression was calculated using the $2^{-\Delta\Delta CT}$ method. All experiments were repeated three times.

Statistical Analysis

Data are presented as the mean ± s.d. from at least three independent experiments. The data were assessed using Graphpad Prism 5.0 software (Graphpad, La Jolla, CA, USA) and SPSS 20.0 software (IBM, New York, NY, USA). Comparisons between groups for statistical significance were performed with a two-tailed paired Student's *t*-test. Correlations between clinical characteristics and miR-655-3p expression were evaluated using the Chi-squared test. Kaplan-Meier analysis and the log-rank test were performed to identify survival differences in HCC patients. Prognostic significance of each variable to overall survival was analyzed using the Cox regression model. $p < 0.05$ was used to indicate a statistically significant difference.

Results

miR-655-3p Expression is Decreased in HCC Tissues

To determine whether miR-655-3p was aberrantly expressed in HCC tissues, qRT-PCR was performed to detect the expression levels of 188 paired clinical HCC tissues and adjacent normal tissues. Our results showed that the average expression level of miR-655-3p was significantly lower in HCC than that in matched normal tissues (Figure 1). These data suggested that miR-655-3p may play a negative effect in the progression of miR-655-3p.

Association Between Clinicopathological Characteristics and miR-655-3p Expression

To further establish the correlation of miR-655-3p expression with clinical prognosis, we manually divided the HCC patients into two groups (high group and low group) according to the average expression of miR-655-3p. The correlations between miR-655-3p expression and the clinicopathological characteristics of the patients are presented in Table I. The results showed that

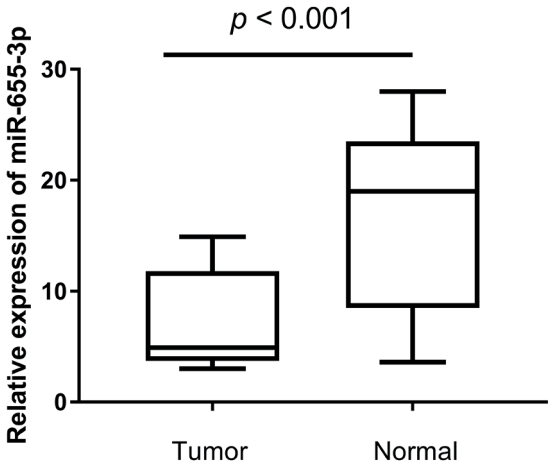

Figure 1. qRT-PCR analysis of miR-655-3p expression in HCC tissues and matched adjacent normal tissues. miR-655-3p was significantly downregulated in HCC tissues.

Table I. Association between miR-655-3p expression and clinicopathological parameters of HCC.

Clinical features	N	miR-655-3p Low (n = 96)	miR-655-3p High (n = 92)	p
Sex				0.153
Male	72	32	40	
Female	116	64	52	
Age (years)				0.254
≥60	119	57	62	
<60	69	39	30	
HBsAg status				0.374
Positive	90	49	41	
Negative	98	47	51	
Tumor size (cm)				0.114
≥5	109	61	48	
<5	79	35	44	
Tumor nodes				0.901
Multi	87	44	43	
Single	101	52	49	
Serum AFP (ng/dl)				0.793
≥200	104	54	50	
<200	84	42	42	
Microvascular invasion				0.000
Yes	109	68	41	
No	79	28	51	
Tumor stage				0.006
I+II	89	36	53	
III+IV	99	60	39	
Lymph node metastasis				0.001
No	98	39	59	
Yes	90	57	33	

miR-655-3p expression was significantly related to microvascular invasion (p = 0.000), tumor stage (p = 0.006), and lymph node metastasis (p = 0.001). However, there were no significant correlations between miR-655-3p expression and other clinicopathologic features, such as age, HBsAg status, or tumor size. Our findings supported the notion that miR-655-3p down-regulation may be associated with tumor progression.

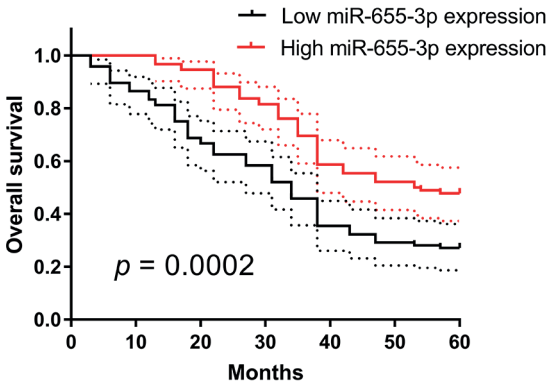

Figure 2. Kaplan-Meier curves of survival in patients with HCC.

Down-Regulation of miR-655-3p Confers Poor Prognosis in HCC Patients

To further analyze the significance of miR-655-3p in terms of clinical prognosis, we performed the log-rank test and the results showed that the overall survival of HCC patients with low miR-655-3p expression was significantly shorter than those with high miR-655-3p expression (p = 0.0002; Figure 2). In the univariate analysis, microvascular invasion (HR = 2.451, 95% CI: 1.113-4.583, p = 0.006), tumor stage (HR = 3.315, 95% CI: 1.776-6.451, p = 0.003), lymph node metastasis (HR = 1.771, 95% CI: 1.021-3.668, p = 0.002), and expression of miR-655-3p (HR = 1.628, 95% CI: 1.144-4.337, p = 0.001) were associated with poor survival. Further multivariate COX regression analysis confirmed that miR-655-3p expression functioned as predictors of poor prognosis (HR=1.533, CI: 0.988-3.891, p = 0.002, shown

Table II. Univariate and multivariate analyses of prognostic factors in HCC patients.

Variables	Univariate analysis HR	95% CI	p	Multivariate analysis HR	95% CI	p
Sex	1.334	0.615-1.931	0.247	1.033	0.514-1.673	0.316
Age	1.517	0.933-2.661	0.193	1.316	0.732-2.016	0.215
HBsAg status	2.416	1.533-2.991	0.511	1.933	1.237-2.144	0.616
Tumor size	1.158	0.812-1.563	0.177	1.422	0.933-2.117	0.217
Tumor nodes	2.166	1.347-3.351	0.183	1.892	1.231-3.033	0.215
Serum AFP	2.655	1.235-3.884	0.214	2.135	1.144-3.235	0.188
Microvascular invasion	2.451	1.113-4.583	0.006	2.013	0.933-3.114	0.008
Tumor stage	3.315	1.776-6.451	0.003	2.674	1.993-5.113	0.005
Lymph node metastasis	1.771	1.021-3.668	0.002	1.417	1.432-4.166	0.006
miR-655-3p	1.628	1.144-4.337	0.001	1.533	0.988-3.891	0.002

in Table II). Taken together, miR-655-3p expression was an independent predictor for overall survival.

Discussion

HCC is one of the most common and aggressive solid organ tumors in many countries[1]. Although the therapeutic approach for HCC has changed significantly in the past decade, the prognosis of HCC patients remains poor[14]. Investigating new therapeutic modalities and identifying prognostic biomarkers for HCC may help improve the therapy methods. Recently, more and more evidence showed that miRNAs served as a tumor promoter or tumor suppressor in all types of cancer by regulating their targeting genes. Shao et al[15] reported that elevated expression of miR-519a was observed in HCC tissues, and overexpression of miR-519a promotes proliferation and inhibits apoptosis of hepatocellular carcinoma cells by targeting FOXF2. Huang et al[16] showed that miR-663a distinctly inhibited HCC cell proliferation, migration and invasion by targeting HMGA2. Zheng et al[17] revealed that ectopic expression of miR-216b produced a suppressive effect on the growth of HCCC cells by targeting Forkhead box protein M1. Those findings provide the support that miRNAs may be used to predict the prognosis of tumor patients.

Several researches have reported that miR-655-3p function as a tumor suppressor in some tumors. For instance, Wang et al[18] found that miR-23b is highly downregulated in human esophageal squamous cell carcinoma and its overexpression suppresses cell invasion by targeting pituitary tumor-transforming gene-1. Lv et al[19] reported that the down expression of miR-655-3p was associated with enhanced metastasis and invasion of breast cancer cells. Harazono et al[20] indicated that miR-655 was significantly decreased in esophageal squamous cell carcinoma cell lines. They further found that a significant correlation between miR-655 expression and a better prognosis in esophageal squamous cell carcinoma, and miR-655 could be a novel EMT-suppressive miRNA. Wu et al[13] provided evidence that The expression of miR-655-3p was increased in HCC tissues and cell lines, and downregulation of miR-655-3p is associated with tumor progression. Moreover, they identified ADAM10 as a direct target of miR-655-3p. On the other hand, ADAM10 was an oncogene in HCC[21]. Thus, the above results identified miR-655-3p as an anti-oncogene in HCC. However, to our best knowledge, the role of miR-655-3p to predict clinical outcome of patients with HCC has not been reported.

In the present work, we performed the RT-PCR and found that The expression of miR-655-3p in HCC specimens was lower than adjacent normal tissues. We also found that the downregulation of miR-655-3p may be markedly associated with the advanced tumor progression, such as tumor stage and microvascular invasion, and lymph node metastasis. Furthermore, Kaplan-Meier analysis with the log-rank test showed that patients with high miR-655-3p expression lived shorter than those with low miR-655-3p expression. More importantly, Multivariable Cox proportional hazards regression analysis confirmed that HULC low expression was an independent prognostic factor in patients with HCC.

Conclusions

Our data give preliminary evidence that miR-655-3p might have potential value for predicting poor prognosis in HCC patients. However, the current paper has not elucidated the exact molecular mechanisms of miR-655-3p in HCC. Further investigation was needed.

Conflict of interest

The authors declare no conflicts of interest.

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