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Involvement of miR-454 overexpression in the poor prognosis of hepatocellular carcinoma

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Abstract. – OBJECTIVE: Many studies informed that microRNAs (miRNAs) could function as diagnostic and prognostic indicators in several cancers. The prognostic value of miR-454 in hepatocellular carcinoma has not been investigated.

PATIENTS AND METHODS: A total of 265 patients with HCC were obtained in this retrospective study between June 2009 and July 2014. qPCR was conducted to evaluate the expressed amount of the miR-454. The Kaplan-Meier method was conducted to explore the survival status of HCC patients. The log-rank test was used to analyze differences in survival rates.

RESULTS: The expression of miR-454 was significantly upregulated in HCC tissues compared with adjacent non-cancerous tissues ($p < 0.001$). High levels of miR-454 in HCC tissues were correlated with a low 5-year overall survival (OS) ($p < 0.001$). Moreover, patients with high miR-454 expression had decreased disease-free survival (DFS) ($p < 0.001$). Furthermore, multivariate analysis showed that up-regulation of miR-454 was an independent prognostic factor for both 5-year OS ($p = 0.013$) and 5-year DFS ($p = 0.008$).

CONCLUSIONS: We firstly prove that expression of miR-454 may be a novel and valuable prognostic factor in HCC.

Key Words:
Hepatocellular carcinoma, MicroRNA-454, Quantitative RT-PCR, Disease-free survival, Overall survival.

Introduction

Human hepatocellular carcinoma (HCC) is the fifth most frequent malignancy worldwide, more than 700,000 new HCC cases are diagnosed every year[1-2]. In China, HCC rank is the second leading cause of cancer-related mortality[3]. As an intricate malignant tumor, many different factors make a difference in development and progression of human hepatocellular carcinoma. Hepatitis B virus infection is one of the most important risk factors[4], despite the recent introduction of concomitant temozolomide with radiotherapy. However, the 5-year survival rate continues quite low among patients with HCC at present[5]. Thus, there is urgent and of great interest to find novel diagnostic and prognostic biomarkers for HCC.

MicroRNAs (miRNAs), a class of small non-coding RNA molecules, induce translational repression or degradation and play an important role in the progress of gene expression[6-7]. Furthermore, a single miRNA can play different role in the regulation of cancer progression and make a difference in the expression of may target genes[8]. More and more evidence revealed that miRNAs play critical roles in cell proliferation, apoptosis, migration, invasion and metabolism[9-11]. miR-454 has been found to be up-regulated in colorectal cancer (CRC) tissues and HCC tissues[10-12]. However, Niu et al[13] found that miR-454 expression was downregulated in osteosarcoma tissues. However, whether miR-454 can function as a prognostic biomarker of HCC remains unknown. Therefore, in our study, we focus on the prognostic role of miR-454 in HCC.

Patients and Methods

Patients and Tissue Samples

This work was approved by the Research Ethics Committee of the PLA General Hospital. Written informed consent was obtained from all of the patients. A total of 265 patients who was diagnosed HCC were included in this retrospective study. Fresh samples were snap frozen in liquid nitrogen immediately after surgical removal and stored at –80°C until use. None of

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the patients recruited had undergone preoperative chemotherapy or radiotherapy. The characteristics of patients are presented in Table I.

RNA Extraction and qRT-PCR

Total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's guide. cDNA was colligated according to the manufacturer's protocol (MBI Fermentas, Burlington, ON, Canada). The expression of miR-454 was determined using The TaqMan microRNA assay and TaqMan universal PCR master mix. Moreover, The expression levels of miR-454 were normalized to U6B. Each sample was examined in triplicate. The comparative threshold cycle (Ct) method was used to analyze the data. The expression levels of miR-454 were normalized to U6B.

Statistical Analysis

SPSS software 16.0 (SPSS Inc., Chicago, IL, USA) was used to carry out all computations. Student's *t*-test was applied to calculate the differential expression of miR-454 between HCC tissues and normal tissues. The differences between miR-454 expression and the clinicopathological variables were analyzed using the $\square 2$ test. p-value < 0.05 was considered significant.

Results

Expression Level of miR-454 in HCC Tissues

We quantitated miR-454 expression in 265 pairs of HCC tissues and paired normal adjacent liver tissues. Our results showed that miR-454 was significantly up-regulated in HCC tissues compared with paired adjacent non-cancerous tissues ($p < 0.001$, Figure 1).

Correlation of miR-454 Expression and Clinical Parameters in HCC

To explore the clinical effect of miR-454 in HCC, miR-454 expression was divided into high and low expression groups, according to the me-

Table I. Clinicopathological features and miR-454 expression in HCC.

Variables	Cases (n = 265)	miR-454 expression level		*p*-value
		Low expression	High expression	
Age (years)				0.402
< 55	153	74	79	
≥ 55	112	60	52	
Gender				0.354
Man	136	65	71	
Woman	129	69	60	
HBsAg				0.015
Postive	146	64	82	
Negative	119	70	49	
AFP (ng/ml)				0.031
< 25.0 cm	129	74	55	
≥ 25.0 cm	136	60	76	
Tumor size (cm)				0.001
< 5	113	70	43	
≥ 5	152	64	88	
Histologic grade				< 0.001
High	138	50	88	
Low	127	84	42	
Tumor number				0.09
Solitary	115	65	50	
Multiple	150	69	81	
Vein invasion				0.744
Presence	101	53	48	
Absence	164	81	83	
TNM stage				< 0.001
I-II	148	102	46	
III-IV	117	32	85	

AFP = a-fetoprotein.

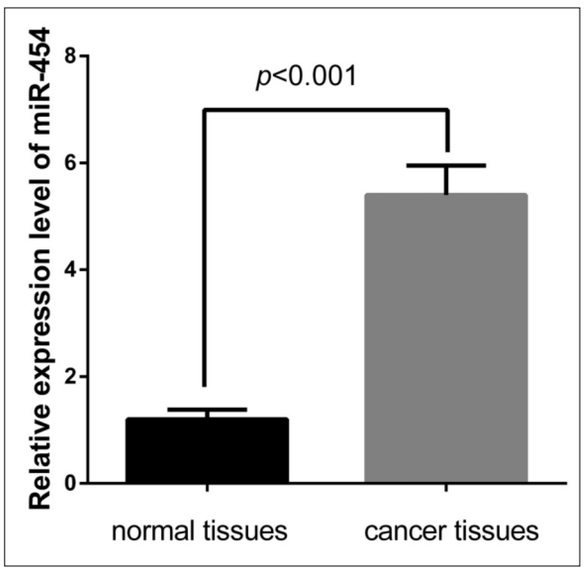

Figure 1. Expression of miR-454 in hepatocellular carcinoma (HCC) and matched adjacent noncancerous liver tissues. The level of miR-454 expression in HCC tissues (n = 265) was higher than that in control tissues (n = 265).

dian expression level of miR-454 in all HCC tissues. The results of statistical analyses between miR-454 and clinicopathological characteristics were summarized in Table I. We found that high miR-454 expression was observed to be significantly associated with AFP level (p = 0.031), HBsAg (p = 0.015), tumor size (p = 0.001), histologic grade (p < 0.001), and high TNM stage (p < 0.001). No significant association of miR-454 expression was found with gender, age, vein invasion, and tumor number (all $p \geq 0.05$, Table I).

Correlation Between miR-454 Expression and Prognosis of HCC Patients

In order to investigate the relationship between miR-454 expression and survival of HCC patients, Kaplan-Meier method and log-rank test were performed to analyze the differences of OS and DFS. As shown in Figure 2, high miR-454 expression had a significant impact on OS (p < 0.001). Moreover, the 5-year DFS rate of HCC patients with high-miR-545 expression was significantly lower than that of patients with low-miR-454 expression (p < 0.001, shown in Figure 3). Multivariate analysis indicated that miR-454 expression independent prognostic factors for OS (p = 0.013), as well as DFS (p = 0.008) in HCC, as shown in Table II.

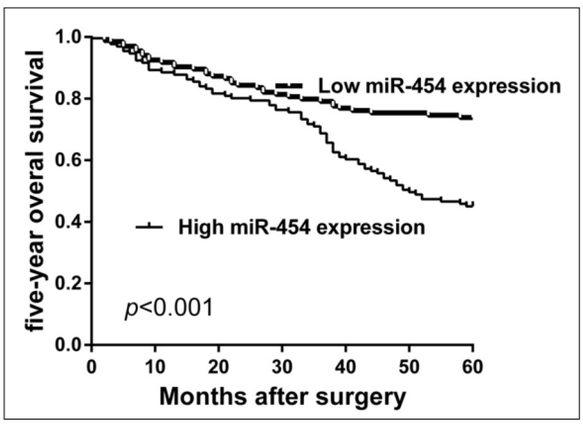

Figure 2. Kaplan-Meier survival curves of patients with hepatocellular carcinoma based on the miR-454 expression. Patients in the high expression group had significantly lower overall survival rates than those in the low expression group (log-rank test, p < 0.001).

Discussion

Many studies have identified miRNAs can function as either oncogenes or tumor suppressors according to their target genes[14-15]. More and more evidence suggest that deregulation of miRNAs influence human carcinogenesis and cancer progression[6-17]. MiR-454 is a relatively newly discovered miRNA, its function has not been clearly characterized.

Recently, some studies reported the role of miR-454 in different tumors, the expression level of miR-454 was up-regulated in colorectal cancer (CRC) tissues and CRC cells. Moreover, ectopic

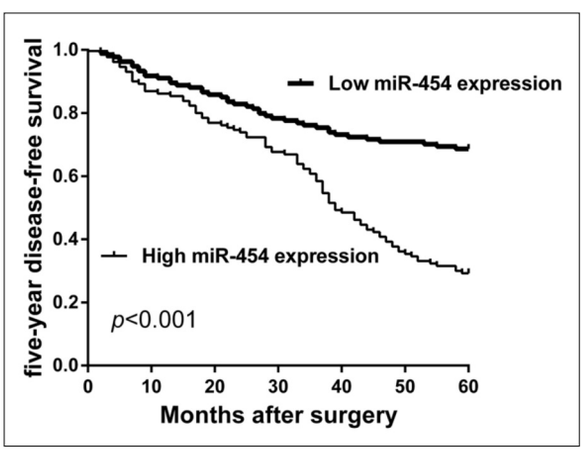

Figure 3. Kaplan-Meier survival curves of patients with hepatocellular carcinoma based on the miR-454 expression. Patients in the high expression group had significantly lower disease-free survival rates than those in the low expression group (log-rank test, p < 0.001).

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

Features	Overall survival		Disease-free survival	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age	0.921 (0.415-1.538)	0.49	0.833 (0.427-1.433)	0.43
Gender	1.186 (0.721-1.454)	0.62	0.881 (0.542-1.676)	0.68
HBsAg	1.532 (0.792-4.384)	0.24	2.241 (0.951-4.871)	0.21
AFP level	2.328 (0.751-5.324)	0.18	3.521 (0.476-11.159)	0.31
Tumor size	3.328 (0.515-6.771)	0.41	4.421 (0.661-9.425)	0.48
Histologic grade	5.132 (2.331-6.221)	0.03	6.129 (3.544-7.881)	0.01
Tumor number	3.211 (0.815-3.318)	0.22	3.231 (0.714-7.221)	0.07
Vein invasion	1.724 (0.722-1.941)	0.11	2.613 (0.618-6.441)	0.15
TNM stage	2.231 (1.212-6.414)	0.006	4.265 (1.451-6.332)	0.004
miR-454 expression	2.719 (1.122-7.229)	0.013	4.412 (1.721-7.445)	0.008

AFP = a-fetoprotein; CI = confidence interval; HR = hazards ratio.

expression of miR-454 play an oncogenic role in human colorectal cancer via targeting Smad4 expression[12]. Perfetti et al[18] showed that up-regulation of miR-454 expression was found in the blood of myotonic dystrophy type 1 patients. Previously, miR-454 expression was observed to be downregulated in osteosarcoma tissues[13], further exploring identified that miR-454 function as suppressor gene in osteosarcoma. Furthermore, Yu et al[10] found that miR-454 served as an oncogene by inhibiting CHD5 in hepatocellular carcinoma and knockdown of miR-454 inhibited the growth of tumor cells in vivo. These results informed that miR-454 expression might be correlated with tumor progression. Especially, miR-454 may serve as an oncogene in HCC. However, no study was reported on the clinical relevance of miR-454 to tumor.

In our present study, we observed that relative expression of miR-454 in HCC tissues is higher than that in matched noncancerous liver tissues. The high expression of miR-454 in HCC tissues was strongly associated with serum AFP level, HBsAg status, tumor size, high histologic grade, and high TNM stage. Our findings informed that miR-454 might contribute to carcinogenesis and metastasis of HCC. Furthermore, we found HCC patients with high miR-454 expression had shorter overall survival than those with low miR-454 expression. Moreover, HCC patients with high miR-454 expression had shorter Disease-free survival than those with low miR-454 expression. Univariate and multivariate analysis confirmed high miR-124 expression as an unfavorable prognostic factor for overall survival and disease-free survival. Therefore, miR-454 could function as an efficient prognostic factor for HCC patients.

Conclusions

This is the first report demonstrating that up-regulation of miR-454 was associated with poorer patients' outcome, implying that it may act as a potential therapeutic target for HCC. A further understanding of the molecular mechanism was needed to develop the novel method of treating HCC.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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