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Upregulation of miR-522 is associated with poor outcome of hepatocellular carcinoma

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Abstract. – OBJECTIVE: The aim of our study was to explore the clinicopathologic and prognostic significance of miR-522 expression in human hepatocellular carcinoma (HCC).

PATIENTS AND METHODS: The expression of miR-522 in 161 HCC tissues and adjacent non-tumor tissues was examined using quantitative real-time-PCR. The association of miR-522 expression with clinicopathological features and the prognosis of HCC patients were also analyzed. The overall survival (OS) was analyzed by log-rank test. Cox regression models were fitted to analyze the effect of prognostic factors on OS.

RESULTS: The relative level of miR-522 was significantly higher in HCC tissues compared to the adjacent normal liver tissues. In addition, miR-522 upregulation more frequently occurred in HCC specimens with lymph node metastasis (p = 0.000), and tumor grade (p = 0.002). Moreover, the level of miR-522 expression was markedly correlated with the HCC patients' overall survival (p < 0.000). In the Cox proportional hazard model, the results showed that miR-522 overexpression was an independent prognostic factor for OS

CONCLUSIONS: Overexpression of miR-522 functions as an unfavorable prognostic biomarker in HCC patients.

Key Words: MiR-522, Hepatocellular carcinoma, HCC, Prognosis.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common highly invasive malignant tumors associated with high incidence and poor prognosis[1,2]. Every year, more than half million of new HCC cases are diagnosed and more than quarter million cancer deaths are found[3]. Although more and more progressions have been made in the treatment of HCC during the recent decades, 5-year survival rate of HCC patients is still not satisfied[4,5]. For advanced HCC patients, some novel molecular targeted therapies can be applied but with limited therapeutic effects. Therefore, the identification of key factors in HCC is important for improving the therapeutic strategy of HCC.

microRNAs (miRNAs) are major factors in the genetic network that regulates a number of significant pathophysiological processes, including the initiation and progression of cancers[6,7]. More and more evidence also shows that cancer is associated with very complex genetic alternations in oncogenes and tumor suppressors. Furthermore, many studies have been noted that the aberrant expression of miRNAs is associated with the prognosis of different human cancer types including HCC[8,9]. Li et al[10] found that up-regulation of MiR-21 and miR-183 can promote growth and invasion of hepatocellular carcinoma (HCC) cells. Zhang et al[11] indicated that low expression of miR-148b predicated a poor prognosis in hepatocellular carcinoma patients. These previous studies provided initial evidence that miRNAs play an important role in cancer progression of HCC.

Up-regulation expression of miR-522 has been found in HCC tissues[12,13]. Zhang et al[14] found that miR-522 functioned as a tumor promoter by targeting DKK1 and SFRP2 in hepatocellular carcinoma. However, to our knowledge, the prognostic value of miR-522 in HCC have not been reported. In this investigation, we focus on the prognostic role of miR-522 in HCC.

Patients and Methods

Patients and Tissue Samples

All tumor and matched normal tissue samples were obtained from 161 HCC patients who underwent routine curative surgery between May

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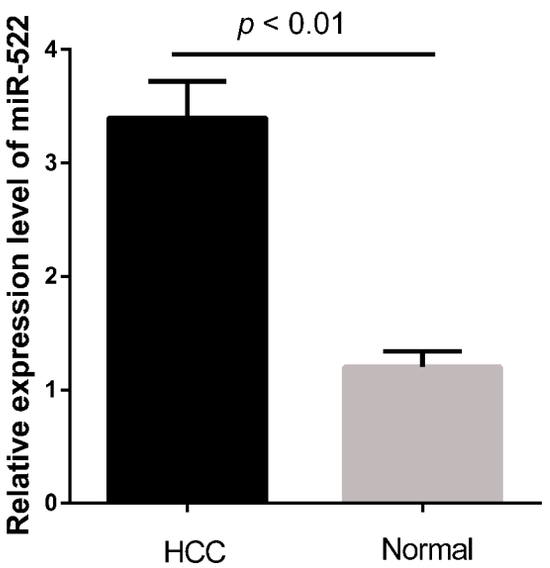

Figure 1. Expression of miR-522 in hepatocellular carcinoma tissues. miR-522 expression was significantly higher in hepatocellular carcinoma tissues than in the corresponding non-tumorous samples ($p < 0.01$).

2008 and July 2014 at the Third People's Hospital of Yancheng City, Jiangsu Province, China. None of the patients had received a preoperative adjuvant therapy. For all cases, the samples were immediately frozen and stored in liquid nitrogen before the analysis. Clinical pathological features, including gender, age, tumor grade and AFP level are listed in Table I. Informed consent was obtained from each patient and the study was approved by the Third People's Hospital of Yancheng City, Jiangsu Province, China.

RNA Isolation and qRT-PCR

Total RNA was extracted from tissues with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer instructions. miRNAs were reverse transcribed to complementary DNA (cDNA). Quantitative mRNA analysis was performed using real-time PCR analysis (TaqMan, PE Applied Biosystems, Foster City, CA, USA). Reaction conditions included: 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 57 °C for 20s, and 72°C for 15s. Relative expression was calculated using the comparative Ct. Each sample was carried out in triplicate. U6 small nuclear RNA was used as an endogenous control.

Statistical Analysis

All statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Wilcoxon-Mann-Whitney test was used to evaluate the relationship between miR-522 expression levels and various clinicopathologic characteristics. OS curves were determined by the Kaplan-Meier method. The multivariate survival analysis was performed using the Cox multivariate analysis model. A two-tailed $p < 0.05$ was considered to indicate a statistically significant difference.

Results

The Expression of miR-522 in the HCC Tissues and the Control Tissues

Levels of miR-522 were measured in 161 snap-frozen HCC tissues samples and adjacent non-cancerous tissues using real-time PCR. The results showed that the miR-522 expression was significantly increased in HCC tissues compared with adjacent non-tumor tissues ($p < 0.01$, as shown in Figure 1), suggesting that miR-522 may play an oncogenic role in HCC.

Correlation of miR-522 Expression with Clinicopathological Characteristics

The associations of miR-522 expression with various clinicopathological parameters of HCC tissues are summarized in Table I. The mean expression level of miR-522 was used to classify the patients with HCC into two groups. High miR-522 expression level was correlated with lymph node metastasis ($p = 0.000$), and tumor grade ($p = 0.002$). However, no significant correlation was observed between miR-522 expression and other clinicopathologic characteristics (all $p > 0.05$).

Association Between miR-522 Expression and Survival in HCC Patients

To explore whether the miR-522 expression can predict prognosis of patients with HCC, we next performed Kaplan-Meier analysis. The results showed that the overall survival rate of HCC patients with high miR-522 expression was significantly lower than that of patients with low miR-522 expression (log-rank test, $p < 0.05$, Figure 2). Furthermore, univariate and multivariate analysis using the Cox proportional hazards model for all variables showed that miR-522 expression was an independent prognostic factor for overall survival ($p = 0.002$, Table II).

Table I. Correlation between miR-552 expression with clinicopathologic features of HCC.

Variable	miR-552 expression		*p*-value
	Low (n = 81)	High (n = 80)	
Ages (years)			0.480
< 50	31	35	
≥ 50	50	45	
Gender			0.577
Male	56	52	
Female	25	28	
Tumor size			0.821
< 5 cm	60	58	
≥ 5 cm	21	22	
Tumor number			0.344
Solitary	51	56	
Multiple	30	24	
Serum AFP (ng/L)			0.796
< 400	33	31	
≥ 400	48	49	
Lymph node metastasis			0.000
No	34	56	
Yes	47	24	
Tumor grade			0.002
G1	40	13	
G2	24	30	
G3	17	37	

Discussion

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death, especially in the face of high WHO tumor grades (III, IV)[15,16]. Despite significant improvements have been achieved in the diagnostics and treatment strategies, clinical outcomes vary significantly between patients and can be difficult to predict. Recently, miRNA studies have highlighted cancer invasion and metastasis. Fan et al[17] found that the decrease expression of miRNA-20a was associated with a poor outcome in HCC patients. Tang et al[18] showed that miR-125a suppressed

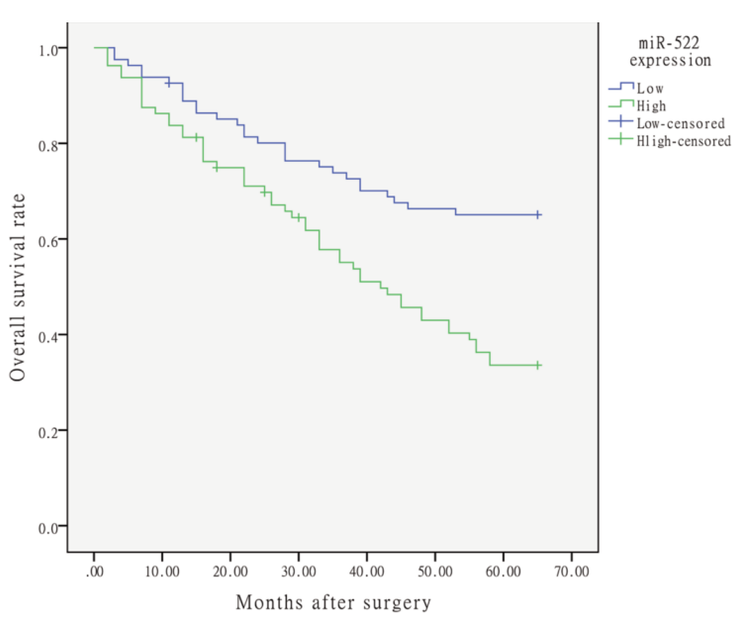

Figure 2. Kaplan-Meier survival curve in relation to miR-522 expression level in patients with epithelial hepatocellular carcinoma. The survival rate of patients with high miR-522 level was significantly lower than of patients with low miR-522 level (log-rank test $p < 0.000$).

Table II. Univariate and multivariate analysis of prognostic parameters in patients with cervical cancers by Cox regression analysis.

	Univariate analysis			Multivariate analysis		
Variables	**HR**	**95% CI**	**p-value**	**Risk**	**95% CI**	***p*-value**
Age (years)	1.62	0.88-1.41	0.19	1.21	0.62-1.31	0.42
Gender	1.37	0.68-2.69	0.27	1.73	1.19-1.66	0.32
Tumor size	1.45	1.25-1.98	0.17	1.65	1.14-3.03	0.28
Tumor Number	1.21	0.87-2.67	0.14	1.39	1.06-2.77	0.24
Lymph node metastasis	1.34	1.04-2.55	0.11	1.47	1.22-3.26	0.14
Tumor grade	2.49	1.03-3.23	0.003	1.54	1.09-3.14	0.009
miR-552	1.45	1.21-2.79	< 0.001	1.37	1.09-5.14	0.002

the migration and invasion of liver cancer cells by targeting PI3K/AKT/mTOR signaling pathway. Those findings indicated that miRNAs might play an important role in HCC initiation and development, and have a great potential for clinical application.

miR-522 encoded 54 tandem miRNAs and normally expressed in a variety of human tissues and deregulated in several types of tumors. Zhang et al[14] found that miR-522 promoted cell proliferation of hepatocellular carcinoma by targeting DKK1 and SFRP2. Zhang et al[19] have discovered that miR-522 is a metastasis promoter in human glioblastoma cells since its increased expression could promote tumor cell proliferation *in vitro*. Furthermore, Zhang et al[20] showed that reduced expression of miR-522 suppressed proliferation and metastasis of non-small cell lung cancer cells by directly targeting DENN/MADD domain containing 2D. All those findings indicated that miR-522 function as a tumor promoter in tumors. However, up to date, the prognostic value of miR-522 in hepatocellular carcinoma has not been reported.

In this investigation, we found that miR-522 was significantly up-regulated in hepatocellular carcinoma tissue compared with adjacent normal tissue. High expression of miR-522 was significantly associated with lymph node metastasis ($p = 0.000$), and tumor grade ($p = 0.002$). Kaplan-Meier analyses show that HCC tissues with high miR-522 expression levels had poorer overall survival. Furthermore, the result of multivariate analyses revealed that miR-522 expression level was independent prognostic factors for overall survival. All those findings indicated the underlying clinical significance of miR-522 overexpression as a biomarker for predicting HCC prognosis.

Conclusions

The expression of miR-522 was significantly increased in HCC patients compared to healthy controls. The survival time of HCC patients with high miR-522 expression is shorter than those with low miR-522 expression. Our findings indicated that miR-522 may be a potential novel target for the gene therapy of HCC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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