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Down-regulation of miR-342-3p in hepatocellular carcinoma tissues and its prognostic significance

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Abstract. – OBJECTIVE: The involvement of microRNAs in cancer and their potential as biomarkers of prognosis are becoming increasingly appreciated. The aim of this study was to evaluate the clinical importance and prognostic value of miR-342-3p in hepatocellular carcinoma (HCC).

PATIENTS AND METHODS: RT-PCR was used to detect the expression of miR-342-3p. The association with clinicopathologic features was analyzed. Kaplan-Meier survival analysis and Cox proportional hazards analysis were used to compare the overall survival between HCC patients with high miR-342-3p expression and those with low miR-342-3p expression.

RESULTS: We found that miR-342-3p expression was significantly decreased in HCC tissues compared with paired adjacent non-tumor tissues ($p < 0.001$). MiR-342-3p expression was correlated with histologic grade ($p = 0.008$) and tumor TNM stage ($p = 0.001$). Kaplan-Meier survival analysis showed that patients in the high miR-342-3p expression group had better overall survival than those in the low miR-342-3p expression group ($p < 0.001$). Univariate analysis showed that miR-342-3p ($p = 0.001$), TNM stage ($p = 0.002$) and histologic grade ($p = 0.006$) were associated with poor survival rates. Multivariate analysis confirmed that miR-342-3p expression can be used as an independent predictor for HCC prognosis ($p = 0.002$).

CONCLUSIONS: miR-342-3p may serve as a tumor suppressor during HCC progression, and its low expression may be a potential biomarker for poor prognosis of HCC.

Key Words:
miR-342-3p, Hepatocellular carcinoma, Prognosis.

Introduction

Hepatocellular carcinoma (HCC) is a very aggressive cancer with a high mortality, and remains one of the most common solid tumors worldwide[1,2]. It was reported that one-half of the new HCC cases and HCC deaths worldwide occurred in the People's Republic of China[3]. Despite recent advances in the diagnosis and treatment of HCC, The 5-year survival rate for HCC patients was poor because of delayed diagnosis of HCC and lack of effective therapy for advanced HCC[4]. Thus, it is important to explore highly sensitive biomarkers that can precisely predict the prognosis and recurrence

MicroRNAs (miRNAs) are small (19–24nt), single-stranded, noncoding RNAs, which can regulate gene expression posttranscriptionally[5,6]. More and more previous investigations have proved that miRs are involved in the initiation, development, and progression of cancers[7,8]. Functional studies have predicted that miRNAs can control the activity of over 30% of all protein-coding genes. Some miRNAs has been identified to serve as either oncogenes or tumor suppressors[9]. For instance, Dou et al[10] showed that miR-187-3p inhibited the metastasis and EMT in HCC by targeting S100A4. Li et al[11] found miR-21 and miR-183 promote HCC proliferation and metastasis by targeting SOCS6. Based on these findings, some researchers hypothesized miRNAs may be used as a candidate factor for predicting prognosis for HCC. Recently, many studies focused on the prognostic value of miRNAs in tumors.

A previous study[12] showed that miR-342-3p may function as a tumor suppressor in HCC. However, to our best knowledge, the prognostic significance of miR-342-3p in HCC has not been reported. In the present work, we investigated whether miR-342-3p expression was associated with the outcome of HCC patients.

Patients and Methods

Patients and Tissue Samples

We enrolled 184 HCC patients in the 302 Military Hospital between July 2009 and September

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2011 in this study. All tumor tissues and normal matched tissues were obtained from the resected tumors and adjacent normal liver tissues of the patients, and were immediately frozen in liquid nitrogen and stored at -80°C until use. None of the patients had undergone preoperative intervention therapy or chemotherapy. Detailed demographic and clinicopathological data such as age, gender, tumor size, liver cirrhosis, tumor number, AFP, hepatitis B, histologic grade and TNM stage. were gathered and summarized. Overall survival was defined as the interval between the dates of surgery and death. Ethical approval was obtained from the 302 Military Hospital Research Ethics Committee, and written informed consent was obtained from each patient.

miRNA Extraction and Quantitative Real-time PCR

Total miRNA from surgical HCC tissues was extracted using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. After total RNA had been extracted, miRNA-specific reverse transcription and PCR amplification were performed. PCR reactions were performed by using SYBR®-Green I master mix (Eurogentec, Ougrée Seraing, Belgium) and were run on a MyiQ™ iCycler (Bio-Rad, Hercules, CA, USA). U6 small nuclear or β-actin RNA were used as internal controls. The fold expression changes between groups were measured using the comparative Ct method ($2^{-\Delta\Delta CT}$). PCR primers for miR-342-3p, U6 and β-actin were purchased from Invitrogen (Foster City, CA, USA).

Statistical Analysis

The data were analyzed with the Statistical Package for the Social Science SPSS 19.0 software (SPSS Inc., IBM, Chicago, IL, USA). The association between miR-342-3p expression and clinicopathological parameters was evaluated by Fisher's exact test. The survival curve was assessed by the Kaplan-Meier method, and the two groups were compared by the log-rank test. Independent prognostic factors were determined using the Cox regression model for multivariate analysis. Differences were considered statistically significant when p was less than 0.05.

Results

MiR-342-3p was Lowly Expressed in HCC

To explore the potential role of miR-342-3p in HCC, expression of miR-342-3p in HCC determined the by real-time RT-PCR. As shown in Figure 1, HCC patients exhibited the lowest and highest expression of miR-339-5p. These results suggested that miR-342-3p plays an anti-oncogenic role in HCC.

MiR-342-3p Expression and Clinicopathological Factors in HCC

Next, we determined whether miR-342-3p expression levels are associated with specific clinicopathological characteristics. Patients were divided into two groups: high and low miR-342-3p expression groups according to the median expression level of miR-342-3p in HCC tissues. Our data showed that miR-342-3p expression was correlated with histologic grade (p = 0.008) and tumor TNM stage (p = 0.001) (Table I). However, miR-342-3p expression was not associated with other parameters such as gender, age, tumor size and liver cirrhosis (p > 0.05, respectively, Table I).

Impact of miR-342-3p Expression on Overall Survival in HCC Patients

To determine the prognostic value of the miR-342-3p expression in human HCC, we performed a Kaplan-Meier survival analysis, and the results revealed that the overall survival rate of patients in the low miR-342-3p expression group was significantly poorer than those of patients in the high miR-342-3p expression group (p < 0.001, Figure 2). To further determine whether miR-342-3p was an independent prognostic factor for HCC, we performed univariate and multivariate analyses. The results indicated that miR-342-3p expression (HR=2.231, 95% CI: 1.234-4.893, p =

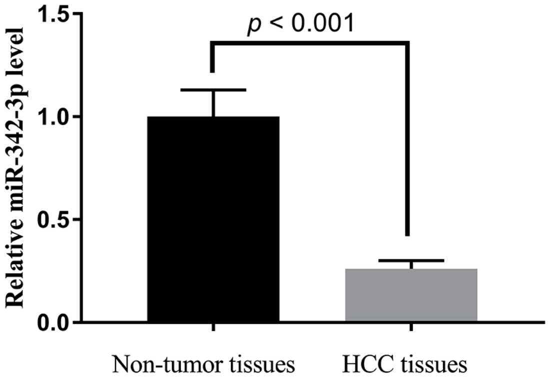

Figure 1. MiR-342-3p expression levels in HCC samples were significantly lower than those in adjacent non-tumor tissues (p < 0.001).

Table I. The association between miR-342-3p expression and clinico-pathological features.

			miR-342-3p expression		
Parameters	**Group**	**Total**	**High**	**Low**	***p* value**
Age (years)	< 60	95	51	44	0.268
	≥ 60	69	69	38	
Gender	Male	111	59	52	0.243
	Female	53	23	30	
Tumor size (cm)	< 5 cm	73	33	40	0.271
	≥ 5 cm	91	49	42	
Liver cirrhosis	Negative	23	14	9	0.261
	Positive	141	68	73	
Tumor number	Solitary	80	39	41	0.755
	Multiple	84	43	41	
AFP	< 20	63	33	30	0.630
	> 20	101	49	52	
Hepatitis B	Negative	49	22	27	0.394
	Positive	115	60	55	
Histologic grade	High	128	71	57	0.008
	Low	36	11	25	
TNM stage	I-II	113	66	47	0.001
	III-IV	51	16	35	

Table II. Univariate and multivariate analysis of overall survival in HCC patients (no. = 164).

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (years) < 60 vs. ≥ 60	0.913	0.566-1.432	0.277	-	-	-
Gender Male vs. Female	1.167	0.811-1.459	0.179	-	-	-
Tumor size < 5 cm vs. ≥ 5 cm	1.633	0.782-1.982	0.163	-	-	-
Liver cirrhosis Negative vs. Positive	1.558	0.475-2.021	0.188	-	-	-
Tumor number Solitary vs. Multiple	2.331	0.713-3.893	0.217	-	-	-
AFP (ng/ml) < 20 vs. > 20	2.133	0.783-2.566	0.133	-	-	-
Hepatitis B Positive vs. Negative	1.893	0.781-2.893	0.211	-	-	-
Histologic grade Low vs. High	2.983	1.144-4.477	0.006	2.231	1.022-3.893	0.009
TNM stage I-II vs. III-IV	3.314	1.778-6.134	0.002	2.893	1.514-5.133	0.006
miR-342-3p expression High vs. Low	2.663	1.547-5.574	0.001	2.231	1.234-4.893	0.002

0.002) was significant prognostic factors for HCC patients (Table II).

Discussion

As the most common primary liver cancer, HCC is the third most frequent cause of cancer-related mortality[13]. Thus, there is an urgent need to identify new molecular targets for the diagnosis, prognosis, and treatment of pancreatic cancer. Although some clinical features of HCC, such as vascular invasion and tumor multifocality, could be used to evaluate the prognosis of HCC patients, it can't satisfied the growing clinical need for highly effective, accurate progno-

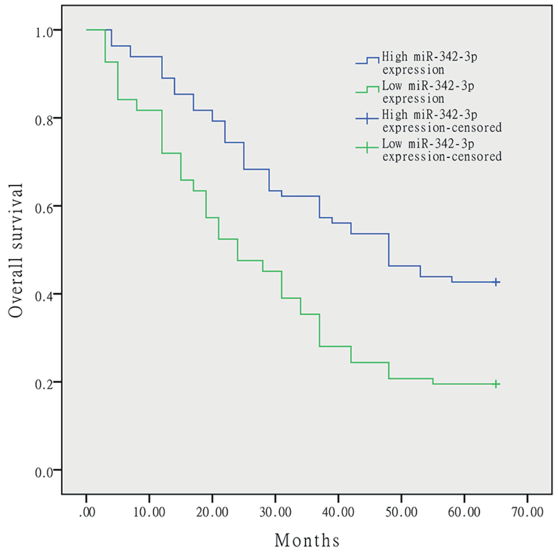

Figure 2. Kaplan-Meier postoperative survival curve for patterns of patients with HCC and miR-342-3p expression.

stic methods for HCC[14,15]. Increasing data revealed that miRNAs may be promising prognostic biomarkers for HCC because of their stability in patients tissues[16,17]. In the present study, our attention focused on miR-342-3p.

The effects of miR-342-3p have been examined in multiple animal models, human cancers, and cell culture systems[18]. Li et al[19] showed that miR-342-3p expression was down-regulated in cervical cancer, and overexpression of miR-342-3p suppresses proliferation, migration, and invasion by targeting FOXM1 in human cervical cancer. Xie et al[20] found that miR-342-3p served as a tumor suppressor in lung cancer. They further identified that the tumor suppressor role of miR-342-3p is mediated through downregulation of RAP2B. On the contrary, another study by Tao et al[21] indicated that the high levels of miR-342-3p were significantly associated with worse survival time. Those data suggested that miRNAs act as oncogenes and/or tumor suppressor genes according to different types of tumors. Recently, Zhao et al[12] found that there was a negative correlation between miR-342-3p expression and the proliferation potential in HCC cells. In *in vivo* experiments, they showed that overexpression of miR-342-3p could suppress HCC cell proliferation via regulating NF-kB pathway. The above results indicated that miR-342-3p may play an important role in the progression of HCC. Therefore, we suggested that miR-342-3p may influence the poor prognosis and progression in HCC.

In the present work, we explored the expression levels of miR-342-3p in HCC patients. The results of PCR showed that the expression levels of miR-342-3p in human HCC tissues were significantly decreased than in the paired normal tissues. The results were consistent with a previous report[12]. Then, we found that miR-342-3p expression was correlated with histologic grade and tumor TNM stage. More importantly, we performed the Kaplan-Meier method and found that patients in the high miR-342-3p expression group had better overall survival than those in the low miR-342-3p expression group. Furthermore, multivariate Cox analysis showed that miR-342-3p could serve as an independent prognostic biomarker. Therefore, miR-342-3p may be a candidate biomarker for predicting clinical outcomes in HCC patients.

Conclusions

This paper is the first study exploring the prognostic value of miR-342-3p in HCC. miR-342-3p levels could be used as an important prognostic marker of clinical outcome in HCC patients.

Conflict of interest
The authors declare no conflicts of interest.

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