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MiR-370 functions as prognostic marker in patients with hepatocellular carcinoma

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Abstract. – OBJECTIVE: Large body of evidence has shown that microRNAs (miRNAs) have the potential to serve as prognosis marker. This study is designed to investigate the expression of miR-370 in hepatocellular carcinoma (HCC) patients and to analyze its potential prognostic value.

PATIENTS AND METHODS: Quantitative real-time quantitative PCR (qPCR) was performed to examine the miR-370 level in HCC tissue and matched normal tissue from 83 HCC patients. The correlation of miR-370 level in HCC tissue with the clinicopathological characteristics was analyzed. Moreover, the Kaplan-Meier method and multivariable Cox regression was utilized to analyze survival data and independent prognostic factors, respectively.

RESULTS: Our results showed that low miR-370 level significantly correlated with tumor node metastasis stage (p=0.013) and vein invasion (p=0.0082). However, no significant relation was found between miR-370 expression and gender (p=0.1275), age (p=0. 0915), size of tumor (p=0.0823), liver cirrhosis (p=0.2508) and tumor grade (p=0.5377). Moreover, addition, survival analysis suggested that low expression of miR-370 linked shorter overall survival compared with high expression and multivariate Cox proportional hazards analysis also showed that miR-370 function as an independent prognostic marker.

CONCLUSIONS: Low level of miR-370 correlates with poor prognosis and miR-370 level can be considered as an independent prognostic marker in clinical evaluations.

Key Words
miR-370, Hepatocellular carcinoma, Prognosis.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignances and is estimated to be the third leading cause of cancer death worldwide[1]. Because of short of sensitive tools for early diagnosis and aggressive nature of HCC, a large number of patients were diagnosed a later stage[2]. Despite significant efforts in improving treatment regimens, most of HCC patients die within one year after diagnosis due to the high occurrence of tumor recurrence and metastasis even after hepatectomy and lack of effective adjuvant therapy[3]. Therefore, identification of novel biomarker that can be used in prediction of tumor recurrence or prognosis of HCC patients are urgently needed[4].

MicroRNAs (miRNAs), a family of small non-coding RNAs, function as either oncogenes or tumor suppressors and regulate a variety of biological activities, such as cell differentiation, proliferation, apoptosis, migration and invasion through repressing the expression of target genes[5]. Accumulating evidence has shown that the miR-370 is commonly down-regulated in a variety of human malignancies, including ovarian cancer[6], gastric cancer[7], liver cancer[8] and non-small cell lung cancer[9] and play regulatory role in proliferation of various cancerous cells, cell differentiation, apoptosis, and chemosensitivity. In the context of HCC, it has been found that miR-370 level was significantly down-regulated in liver cancer cells and functions as tumour suppressor[8]. However, the prognostic value of miR-370 expression in HCC has not been investigated. In present study, miR-370 expression was examined by in HCC patients with long-term follow-up to explore the prognostic role of miR-370.

Patients and Methods

Patients and Tissues Samples

Between September 2011 and March 2016, HCC tissue samples and matched normal tissues were collected from a total of 83 patients who had undergone curative surgery without preoperative adjuvant therapy at the Second Affiliated Hospi-

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Table I. Correlation between miR-370 level and clinicopathological characteristic of HCC patients.

Feature	Cases	miR-370 expression		*p*-value
		Low (n=47)	High (n=36)	
Sex				
Female	25	11	14	0.1275
Male	58	36	22	
Age				
> 50	49	24	25	0.0915
≤ 50	34	23	11	
Tumor size				
> 5 cm	39	26	13	0.0823
≤ 5 cm	44	21	23	
Vein invasion				
Negative	59	28	31	0.0082**
Positive	24	19	5	
Liver cirrhosis				
Negative	11	3	5	0.2508
Positive	72	44	31	
Histological grade				
G1	23	13	10	0.5377
G2	32	16	16	
G3	28	18	10	
TNM stage				
I+II	48	20	28	0.0013*
III+IV	35	27	8	

Note: *p<0.05, **p<0.01.

tal of Baotou Medical College. The present study protocol was approved by Medical Ethics Committee of the Second Affiliated Hospital of Baotou Medical College and written consent forms were obtained from all patients. The tissues specimen was snap frozen and kept in liquid (-70 °C) until analysis. All samples were blindly examined by two senior pathologists. Clinicopathological characteristics of HCC patients were presented in Table I. Overall survival was defined as the time between the surgery and death. Follow-up of all included patients were performed by telephone and a written form. Death cases were reported by family member or carer of patients.

Quantitative Real-time PCR

TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to extract total RNA from human tissues. miR-370 expression was quantified by real time PCR with a TaqMan Probe (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Briefly, cDNA was obtained by High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) and qRT-PCR was performed using a TaqMan PCR kit and the ABI 7500 System (Thermo Fisher Scientific, Waltham, MA, USA). The relative expression of miR-370 tissues was normalized to that of U6.

Statistical Analysis

In present study, the statistical analysis was conducted using SPSS software 16.0 (SPSS Inc., Chicago, IL, USA). The comparison of miR-370 levels in tumor and normal tissue were performed using student's t test. The association between miR-370 levels and clinicopathological characteristics was analyzed by Fisher's test. The survival data was analyzed using the log-rank test and Kaplan-Meier method. Moreover, independent factors relevant to patient survival were analyzed using a Cox proportional hazards modeling. p<0.05 was considered as statistically significant.

Results

Comparison of miR-370 Levels in HCC and Matched Normal Tissues

The miR-370 levels in tissue were determined using quantitative real-time PCR. As shown in Figure 1, decreased level of miR-370 was ob-

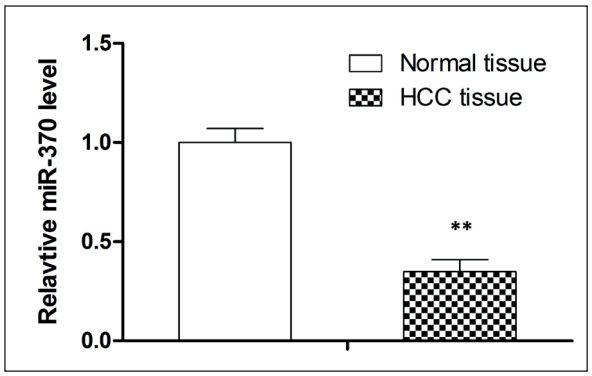

Figure 1. MiR-370 level in HCC tissues. **p<0.01 vs. control.

served in HCC tissue compared to matched normal tissue (p<0.01), suggesting that miR-370 may act as tumor suppressor in HCC.

Correlation Between miR-370 Levels a nd Clinicopathological Characteristics

According to the miR-370 expression data, patients with HCC were assigned to low and high expression groups based on the mean expression level of miR-370. A total of 47 patients were assigned to the low miR-370 expression group while 36 patients were assigned to the high expression group. Following grouping, the association of miR-370 level with clinicopathological characteristics was analyzed. As presented in Table I, low miR-370 level significantly correlated with tumor node metastasis (TNM) stage (stages III and IV; p=0.013) and vein invasion (p=0.0082). However, no significant relation was found between miR-370 expression and gender (p=0.1275), age (p=0.0915), size of tumor (p=0.0823), liver cirrhosis (p=0.2508) and tumor grade (p=0.5377). Given the correlation of miR-370 with tumor node metastasis and vein invasion, miR-370 might play an important role in inhibiting HCC metastasis.

Correlation Between miR-370 Level and Survival of HCC Patients

The association between miR-370 level and survival of HCC patients was analyzed using Kaplan-Meier method. As shown in Figure 2, low miR-270 expression was remarkably associated with shorter overall survival (in log-rank test, p=0.037). Then the median survival time was compared between low and high miR-370 groups. As shown in Figure 2, the median survival time of patients in low miR-370 group was 34.5 months (95% CI 23-44) while high miR-370 expression has a significantly elongated median survival of 44.6 months

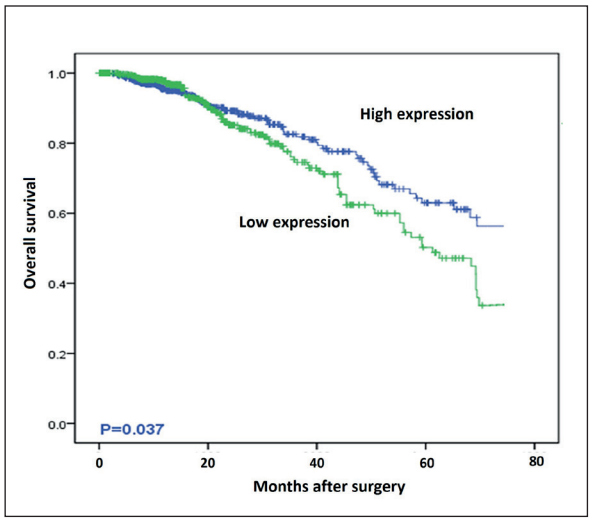

Figure 2. Correlation between miR-370 and survival of HCC patients.

(95% CI 38-54). Next, multivariate Cox regression analysis was employed to examine the prognostic value of the miR-370 and other parameters in HCC. As presented in Table II, our results suggested that low miR-370 expression, TNM stage, and vein invasion were independently predicted poor outcome of patients with HCC whereas other factors including sex, age, tumor size and histological grade had no significant prognostic value for overall survival of HCC patients.

Discussion

microRNAs (miRNAs), accounting for a family of small non-coding RNAs with a length of 21-25 nucleotides, play an important role in a variety physiological activities of cells, including pro-

Table II. Prognostic factors for overall survival by multivariate analysis.

Variable	HR	95 % CI	p-value
Sex	0.954	0.621-2.965	0.752
Age	1.321	0.891-3.158	0.865
TNM stage	2.568	1.425-8.692	0.019*
Tumor size	1.545	1.118-3.852	0.218
Vein invasion	2.698	1.485-7.965	0.026*
Histological grade	1.369	0.658-3.697	0.322
miR-370 expression	2.365	1.015-9.324	0.016*

Note: *p<0.05.

liferation, development, apoptosis, and differentiation through post-transcriptionally regulating expression of their target genes[10]. Accumulating evidence has showed that the aberrant up-regulation or downregulation of miRNAs correlates with the development and prognosis of different human malignancies including HCC[11]. For instance, downregulation of miR-148b has been found to correlate with poor outcome in HCC[12]. Shi et al[13] reported that elevated level of miR-522 predicts poor prognosis in patients with HCC. In the present work, the miR-370 expression in HCC tissue was determined with qRT-PCR and compared with that of normal tissues. Our results showed that miR-370 is abnormally downregulated in HCC tissues and functions as tumor suppressor.

Although accumulating evidence has demonstrated that miR-370 may function as an oncogene or a tumor suppressor gene. For instance, miR-370 was aberrantly downregulated and associated with poor prognosis in laryngeal squamous cell carcinoma, *Helicobacter pylori*-induced gastric carcinoma, and acute myeloid leukemia[7,14,15]. In cholangiocarcinoma, ectopic overexpression of miR-370 was reported to exert anti-proliferative effect on cholangiocarcinoma cells[16]. Moreover, restoration of miR-370 was found to resensitize endometrioid ovarian cancer cell to cisplatin[6]. On the other hand, miR-370 plays an oncogenic role in some other human malignancies. For example, increase in miR-370 level resulted in promoting proliferation in the Wilms tumor G401 cell line[17]. In human prostate and gastric cancers, miR-370 was found to be up-regulated and to function as an oncogene by directly regulating FOXO1[18,19]. Lately, the role of miR-370 as oncogenic miRNA has also been identified in breast cancer[20]. In hepatocellular carcinoma cells, miR-370 showed the potential to suppress metastasis by inhibiting migration and invasion[21]. Sun et al[8] also reported that increasing miR-370 expression promotes cell death of liver cancer cells *in vitro*. In the present work, we found that miR-370 was expressed in a significantly lower level in HCC tissues compared with corresponding normal control tissues. Collectively, these results suggest that miR-370 may play a role as tumor suppressor in HCC.

Moreover, the correlation between miR-370 expression with clinicopathological features and survival of cancers cases were investigated in this study. Our findings showed that declined diction of miR-370 was significantly associated with advanced TNM stage (stages III and IV) and vein invasion (p=0.0013 and p=0.0082, respectively), which indicating that miR-370 might correlate with tumor invasion and progression. In addition, results from survival analysis suggested that low expression of miR-370 linked shorter overall survival compared with high expression, suggesting the potential of miR-370 to serve as a prognostic predictor of HCC. Multivariate Cox proportional hazards analysis also showed that miR-370 function as an independent prognostic marker, which indicated that repressed expression of miR-370 was associated with shorter survival of HCC patients.

Conclusions

We observed that miR-370 is aberrantly downregulated in HCC tissue and miR-370 level is significantly related to advanced cancer stage. Moreover, low level of miR-370 correlates with poor prognosis and miR-370 level can be considered as an independent prognostic marker in clinical evaluations.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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