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Original Article

High expression of miR-20a predicated poor prognosis of hepatocellular carcinoma

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Abstract: Background: MicroRNA-20a (miR-20a) has been found to be up-regulated in some types of cancer. However, the associations between miR-20a expression and the clinical features to determine its clinicopathologic significance in hepatocellular carcinoma (HCC) have not been investigated. Methods: Matched specimens of HCC and paracarcinomatous liver tissues were obtained from 95 patients between January 2013 and October 2013. We detected miR-20a expression levels in HCC tissues and adjacent non-cancerous hepatic tissues using real-time RT-PCR. Correlation of miR-20a expression with overall survival was estimated by the Kaplan-Meier method. Multivariate analysis of the prognostic factors was performed with Cox regression model. Results: qRT-PCR showed that the expression level of miR-20a was up-regulated in HCC tissues compared with matched paracarcinomatous liver tissues (P=0.003). We found that increased miR-20a expression was significantly associated with malignant behavior, such as lymph node metastasis (P=0.014), distant metastasis (P=0.031), and TNM stage (P=0.015). Kaplan-Meier survival curves indicated that HCC patients with high miR-20a expression had a significantly shorter survival time (P=0.014). Multivariate Cox regression analysis showed that miR-20a expression level (HR=2.781, 95% CI: 1.665-8.912, P=0.016) was independently associated with poor survival of patients with HCC. Conclusion: Our results suggested that miR-20a expression level was related with malignant process of HCC, and might be use as prognostic marker for HCC.

Keywords: Hepatocellular carcinoma, microRNA-20a, overall survival, prognosis

Introduction

Hepatocellular carcinoma (HCC) ranks as the sixth most common cancer and the second leading cause of cancer-related mortality worldwide [1]. Although recent advances in functional genomics provide a deeper understanding of hepatocarcinogenesis, the molecular pathogenesis of HCC remains rather unclear. Therefore, many researchers devote themselves to find pathogenesis and more effective therapy [2-4].

MicroRNAs (miRNAs) are small non-coding RNAs of approximately 22 nucleotides (nt) and act as post-transcriptional regulators of gene expression via complimentary pairing predominantly to the 3'-untranslated region (3'-UTR) [5]. Many miRNAs have been demonstrated to actively participate in the regulation of tumor development as tumor suppressor genes or oncogenes. Their emerging roles in the development and progression of human cancers may represent novel diagnostic and therapeutic opportunities, and also supply the prognostic information on tumor development [6-9]. MicroRNA-20a (miR-20a) has been found to be up-regulated in some types of cancer, such as gastric cancer, thyroid cancer, prostate cancer, cervical cancer, and glioma [10-12]. Previously, Zhang et al found that miR-20a levels were increased in HCC cell lines and tissues. In addition, miR-20a induced HCC cell radioresistance by activating the PTEN/PI3K/Akt pathway, suggesting that miR-20a/PTEN/PI3K/Akt might represent a target of investigation for developing effective therapeutic strategies against HCC [13]. In the present study, we aimed to investigate the clinical significance and prognostic value of miR-20a in HCC.

Table 1. The relationship between miR-20a expression and clinicopathologic characteristics in 95 HCC patients

Variables	Case (n)	miR-20a expression level		*P* value
		High (n=47)	Low (n=48)	
Gender				
Male	60	27	33	0.292
Female	35	20	15	
Age				
≤ 60	37	21	16	0.297
> 60	58	26	32	
Hypertension				
No	36	19	17	0.675
Yes	59	28	31	
Diabetes mellitus				
No	85	41	44	0.523
Yes	10	6	4	
Tobacco smoking				
No	62	29	33	0.522
Yes	33	18	15	
Alcohol consumption				
No	73	35	38	0.633
Yes	22	12	10	
Tumor size (cm)				
≥ 5 cm	49	28	21	0.152
< 5 cm	46	19	27	
Lymph node metastasis				
Absent	83	37	46	0.014
Present	12	10	2	
Distant metastasis				
Absent	87	40	47	0.031
Present	8	7	1	
Stage				
I/II	66	27	39	0.015
III/IV	29	20	9	

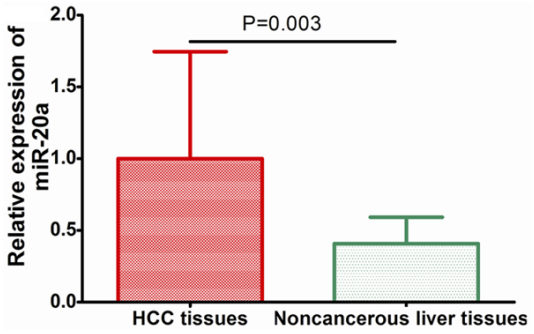

Figure 1. The relative expression level of miR-20a in human HCC tissues and matched adjacent noncancerous liver tissues.

Materials and methods

Patients and tissue samples

The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Dalian Medical University and Dalian Municipal Central Hospital. Informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards. Matched fresh specimens of HCC and paracarcinomatous liver tissues were obtained from 95 patients who underwent hepatic resection at the First Affiliated Hospital of Dalian Medical University and Dalian Municipal Central Hospital between January 2013 and October 2013. All subjects were asked to fill a questionnaire to investigate the demographic characteristics, disease history and the history of cancer and alcohol or tobacco use. The clinical characteristics including tumor differentiation, tumor size, metastasis, Child-Pugh class, chemotherapy and surgery were collected from medical records. Patients with secondary or recurrent tumors, a history of other malignant tumors or being included in other studies were excluded from this study. None of the patients recruited in this study had chemotherapy or radiotherapy before the surgery. Details of clinical and pathological characteristics of the patients are summarized in **Table 1**.

Quantitative real-time reverse-transcription (RT)-PCR

Total RNA was extracted from frozen liver tissues using TriZol reagent (Invitrogen) following

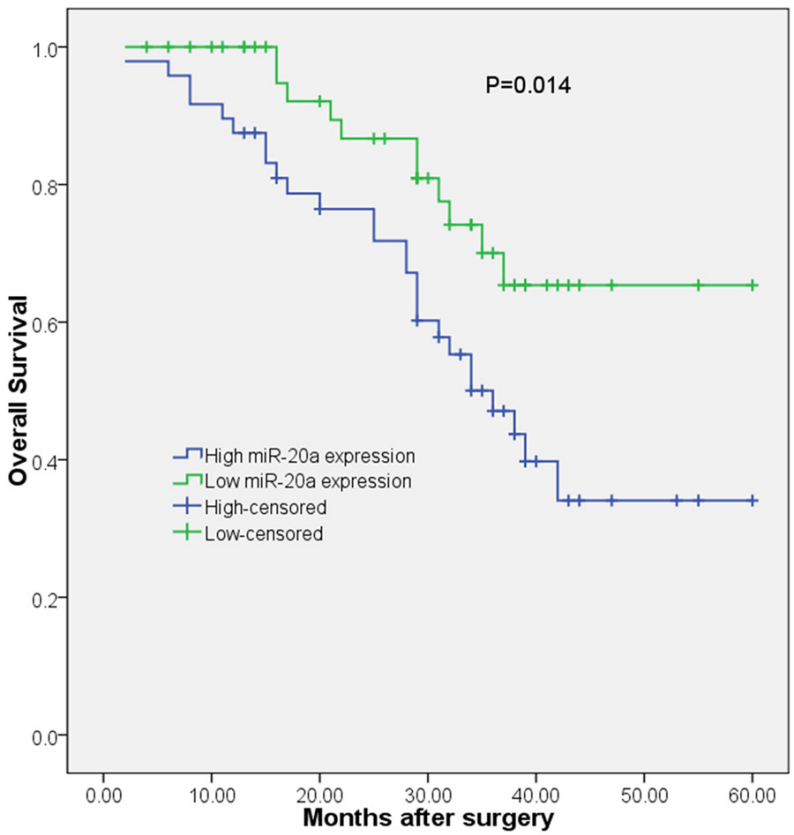

Figure 2. Kaplan-Meier curves of the overall survivals of 95 HCC patients were scored as low expression level and high expression level according to the miR-20a expression.

the manufacturer's instructions. The mature miR-20a was measured by using TaqMan miRNA detection kit (Applied Biosystems, Foster, CA, USA), according to the instructions provided by the manufacturer. RNA samples were reversely transcribed with miRNA-specific primers from TaqMan miRNA Reverse Transcription Kit (Applied Biosystems). Real-time polymerase chain reaction (RT-PCR) was performed with a TaqMan probe, which ensures discrimination of even one nucleotide difference, by using 7300 sequence detection system (Applied Biosystems). The ΔΔCT method was used to determine relative number of copies

(RQ) of miRNA. The U6 was chosen as the endogenous normalizer. Each sample was analyzed in triplicate. The primers were synthesized (Shanghai GenePharma, China) as follows: miR-20a forwards primer: TAC GAT AAA GTG CTT ATA GTG CAG GTA G. U6 forwards primer: ATT GGA ACG ATA CAG AGA AGA TT. Universal reverse primer: GTC CTT GGT GCC CGA GTG.

Statistical analysis

The t-test between two groups was used to analyze the differences between two groups. Correlation of miR-20a expression with overall survival was estimated by the Kaplan-Meier method, and the resulting curves were compared by the log-rank test. Multivariate analysis of the prognostic factors was performed with Cox regression model. SPSS17.0 software for Windows (SPSS Inc, USA) was used for statistical analysis. The *P* values of less than 0.05 were considered to be statistically significant.

Results

miR-20a expression level in HCC tissues

qRT-PCR showed that the expression level of miR-20a was up-regulated in HCC tissues compared with matched paracarcinomatous liver tissues (P=0.003, shown in **Figure 1**). The 95 HCC patients were classified into two groups according to the median of miR-20a expression level as determined by quantitative RT-PCR. 47 cases were placed in the high expression group and 48 in the low expression group.

Correlations of miR-20a expression with clinicopathologic features of HCC patients

We next analyzed the association between the miR-20a expression and various clinicopathological factors of the HCC patients. We found that increased miR-20a expression in HCC was significantly associated with malignant behavior, such as lymph node metastasis (P=0.014), distant metastasis (P=0.031), and TNM stage (P=0.015). However, the expression level was not significantly associated with other clinicopathological factors, including gender, age,

Table 2. Multivariate Cox regression analysis of potential prognostic factors for survival in patients with HCC

Variables	HR	95% CI	*P*-value
Gender	0.572	0.336-1.879	0.476
Age	1.779	0.693-2.463	0.172
Hypertension	1.056	0.687-1.995	0.341
Diabetes mellitus	1.743	0.782-3.031	0.155
Tobacco smoking	1.557	0.622-2.097	0.362
Alcohol consumption	1.774	0.793-3.682	0.267
Tumor size (cm)	2.093	0.781-5.796	0.099
Lymph node metastasis	2.987	1.558-10.023	0.019
Distant metastasis	3.362	1.891-11.723	0.004
TNM stage	3.012	2.101-10.982	0.007
miR-20a expression level	2.781	1.665-8.912	0.016

hypertension, diabetes mellitus, tobacco smoking, alcohol consumption, and tumor size (all *P* value > 0.05, shown in **Table 1**).

miR-20a expression and prognosis of patients with HCC

Kaplan-Meier survival curves indicated that HCC patients with high miR-20a expression had a significantly shorter survival time (P=0.14, shown in **Figure 2**). Multivariate Cox regression analysis showed that lymph node metastasis (HR=2.987, 95% CI: 1.558-10.023, P=0.019), distant metastasis (HR=3.362, 95% CI: 1.891-11.723, P=0.004), TNM stage (HR=3.012, 95% CI: 2.101-10.982, P=0.007) and miR-20a expression level (HR=2.781, 95% CI: 1.665-8.912, P=0.016) were independently associated with poor survival of patients with HCC (shown in **Table 2**).

Discussion

As a highly aggressive solid tumor, HCC is characterized by fast infiltrating growth, early metastasis, high-grade malignancy, and poor prognosis. Curative therapies of surgical treatment, including hepatic resection and liver transplantation, improve the survival of HCC patients greatly. However, the prognosis for most patients remains poor because of multicentric recurrence and intrahepatic metastasis [14-16]. Therefore, more extensive investigations are needed to identify miRNAs which can be employed as prognosis predictor or therapeutic target for HCC.

miRNAs are a class of non-coding small RNAs that regulate expression of genes at post-transcriptional level. They are involved in various biological processes, including development, differentiation, signal transduction and carcinogenesis [17-20]. In recent years, several studies have demonstrated that alterations in miRNA genes lead to tumor formation; several miRNAs which regulate either tumor suppressor or promoter have been identified [21, 22]. Altered miRNA expressions have been identified as modulators of cell growth, apoptosis, migration or invasion in HCC. Therefore, more extensive investigations are needed on the role of miRNAs, which are deregulated in HCC in order to elucidate the function of miRNAs in HCC [23].

miR-20a has been found to be up-regulated in some types of cancer. For example, Du et al found that miR-20a was significantly upregulated in gastric cancer plasma and tissue samples. In addition, miR-20a could promote activation of the NFκB pathway and downstream targets livin and survivin by targeting NFKBIB, which potentially contributed to gastric cancer chemoresistance [24]. In the study by Zhao et al, they detected the relationship between miR-20a and the development of cervical cancer by qRT-PCR, they found that the expression level of miR-20a was significantly higher in cervical cancer patients than in normal controls, and the aberrant expression of miR-20a was correlated with lymph node metastasis, histological grade and tumor diameter. Furthermore, inhibited miR-20a prevented tumor progression by modulating cell cycle, apoptosis, and metastasis in vitro and in vivo, suggesting miR-20a might be used as therapeutic agent for cervical cancer [25]. Qiang et al found that miR-20a was significantly up-regulated in prostate cancer compared with normal prostate tissues, and it significantly contributed to the progression of prostate cancer by targeting ABL2 [12].

Previously, Zhang et al found that miR-20a levels were increased in HCC cell lines and tissues. In addition, miR-20a induced HCC cell radioresistance by activating the PTEN/PI3K/

Akt pathway, suggesting that miR-20a/PTEN/PI3K/Akt might represent a target of investigation for developing effective therapeutic strategies against HCC [13]. In the present study, we aimed to investigate the clinical significance and prognostic value of miR-20a in HCC. qRT-PCR showed that the expression level of miR-20a was up-regulated in HCC tissues compared with matched paracarcinomatous liver tissues. We next analyzed the association between the miR-20a expression and various clinicopathological factors of the HCC patients. We found that increased miR-20a expression in HCC was significantly associated with malignant behavior, such as lymph node metastasis, distant metastasis, and TNM stage. However, the expression level was not significantly associated with other clinicopathological factors, including gender, age, hypertension, diabetes mellitus, tobacco smoking, alcohol consumption, and tumor size. Kaplan-Meier survival curves indicated that HCC patients with high miR-20a expression had a significantly shorter survival time. Furthermore, multivariate Cox regression analysis showed that lymph node metastasis, distant metastasis, TNM stage, and miR-20a expression level were independently associated with poor survival of patients with HCC. In conclusion, our results suggested that miR-20a expression level was related with malignant process of HCC, and might be use as prognostic marker for HCC.

Disclosure of conflict of interest

None.

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