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Original Article

Decreased expression of serum miR-424 correlates with poor prognosis of patients with hepatocellular carcinoma

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Abstract: Background: MicroRNAs (miRNAs) play important roles in many important cellular processes and deregulation of miRNAs is linked to many human diseases including cancer. Although miR-424 has been demonstrated to inhibit progression of hepatocellular carcinoma (HCC), its expression level in serum samples and the potential clinical values remain unknown. Materials and methods: The expression level of miR-424 in the serum clinical samples from HCC patients and healthy volunteers were determined by qRT-PCR. Then the association of serum miR-424 expression level with various important clinicopathological parameters and survival rates was evaluated. Multivariate Cox regression analysis was used to identify the independent risk factors for HCC. Results: The expression level of serum miR-424 was significantly decreased in patients with HCC compared with the healthy volunteers ($P<0.01$). Reduced expression of serum miR-424 was associated with serum AFP ($P=0.048$), vein invasion ($P=0.006$) and TNM stage ($P=0.003$). In addition, survival analysis showed that HCC patients with lower serum miR-424 expression suffered poorer overall survival ($P=0.018$) and disease free survival ($P=0.008$). Moreover, serum miR-424 was demonstrated to be an independent risk factor for HCC. Conclusions: Our findings provide the compelling evidence that the decreased expression of serum miR-424 may serve as a novel biomarker to predict the unfavorable prognosis of HCC patients.

Keywords: Hepatocellular carcinoma, miR-424, prognosis, serum

Introduction

Hepatocellular carcinoma (HCC), a primary malignancy of the liver, is the third leading cause of cancer-related death worldwide [1, 2]. Despite great advance has been made in the field of diagnosis and multi-modality treatments, the clinical outcome of HCC remains unclear [3]. High rates of recurrence and metastasis are the major reasons responsible for the poor prognosis [4]. Therefore, detecting biomarkers that can identify HCC at an early clinical stage and predict the prognosis of HCC might not only help clinical treatments, but also significantly improve the outcome of this malignant disease.

MicroRNAs (miRNAs) are a group of short non-coding, highly conservative RNAs that regulate gene expression at the post-transcriptional level [5]. MiRNAs play important roles in most physiological processes such as cell proliferation, survival and differentiation [6, 7]. Deregulation of miRNAs is closely associated with the initiation and progression of many types of cancers including HCC [8]. Zhai et al showed that miR-129 was down-regulated in HCC tissues and its decreased expression was correlated with tumor progression. In addition, enforced expression of miR-129 suppressed HCC cells proliferation and invasion, while induced apoptosis; indicating miR-129 might function as a tumor suppressor in HCC [9]. Ma et al revealed that the expression level of miR-24 was increased in HCC tissues and cell lines. Moreover, down-regulation of miR-24 could inhibit the proliferation, migration and invasion

Table 1. Correlations between serum miR-204 expression and clinicopathological features in HCC patients

Parameters	n	Serum miR-204 expression		*P*
		Low	High	
Gender				
Male	72	38	34	0.133
Female	23	8	15	
Age				
<50	51	29	22	0.076
≥50	44	17	27	
Tumor size (cm)				
<5	37	16	21	0.420
≥5	58	30	28	
Serum AFP (μg/L)				
<400	65	27	38	0.048
≥400	30	19	11	
Live cirrhosis				
No	17	10	7	0.344
Yes	78	36	42	
Vein invasion				
No	27	7	20	0.006
Yes	68	39	29	
Tumor number				
Single	53	26	27	0.889
Multiple	42	20	22	
TNM stage				
I-II	46	15	31	0.003
III-IV	49	31	18	
Tumor differentiation				
Well+Moderate	43	18	25	0.247
Poor	52	28	24	

capacity of HCC cells by partly targeting sex-determining region Y (SRY)-box 7; suggesting miR-24 might be a positive regulator of HCC progression [10].

Aberrant expression of miR-424 is frequent in a number of cancers including HCC. Previous studies have demonstrated that miR-424 was down-regulated in HCC tissues and cell lines, and it might play a tumor suppressive role in the development of HCC [11, 12]. However, whether miR-424 is up-regulated in serum samples from HCC patients and it has any potential clinical values are poorly known. Thus the aim of the current study was to explore the clinical significance of miR-424 for HCC.

Materials and methods

Study population and clinical samples

The study was approved by the Research Ethnic Committee of The First Affiliated Hospital of Shantou University Medical College. The written inform consent was obtained from all the participants. Serum samples were drawn from 95 patients with HCC and 40 healthy volunteers from the Department of Gastroenterology, The First Affiliated Hospital of Shantou University Medical College. The patients with HCC were pathologically diagnosed and they did not receive any kind of therapy before serum samples collection. The clinical feature of HCC patients was summarized in **Table 1**.

qRT-PCR

Approximately 5 mL of whole blood were collected from each participant. To remove the cellular components completely, the serum was separated by centrifugation at 1,200 × g at 4°C for 20 min and passed through a 13 mm serum filter (Thermo Fisher Scientific Inc.). QIAamp RNA Blood kit (Qiagen, Hilden, Germany) was used to extract total RNA from the cells and then Prime-Script RT reagent kit (TaKaRa, Dalian, China) was employed for reverse transcription and PCR reaction. The PCR reaction was performed on the Applied Biosystems 7500 Fast platform (Applied Biosystems, CA, USA). The average expression level of serum miR-424 was normalized against U6 using the 2-ΔCt method. Each experiment was performed in triplicate.

Statistical analysis

The expression level of serum miR-424 in HCC patients and healthy volunteers was compared by Mann-Whitney U test. Then the correlation between clinicopathological parameters and serum miR-424 expression level was evaluated using χ^2 tests. The Kaplan-Meier method and log-rank test were used to analyze the association between serum miR-424 with OS as well as DFS. The Cox proportional hazards model was employed for the multivariate analysis. Statistical analyses were performed using SPSS 21.0 software (Chicago, Ill., USA) and the $P<0.05$ was indicated to be statistically significant.

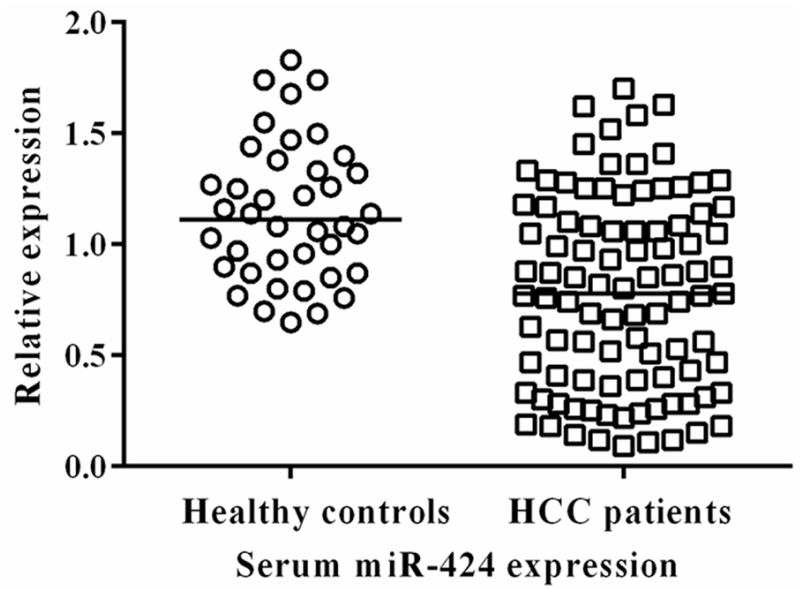

Figure 1. Serum miR-424 was down-regulated in patients with HCC.

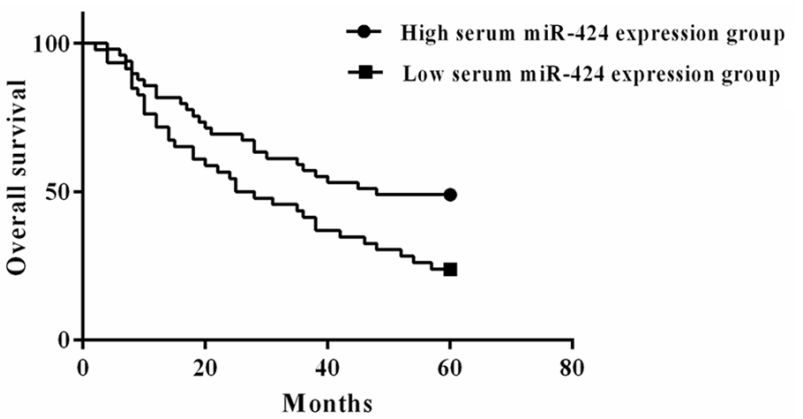

Figure 2. Low serum miR-424 was correlated with poor overall survival.

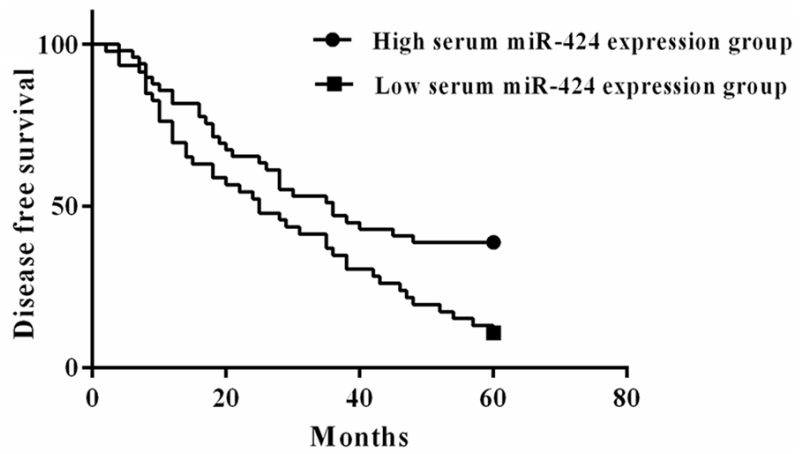

Figure 3. Low serum miR-424 was correlated with poor disease free survival.

Results

Serum miR-424 was down-regulated in patients with HCC

The results showed that the expression level of serum miR-424 was significantly lower in patients with HCC compared with the healthy controls (P<0.01) (**Figure 1**).

Correlations between serum miR-424 and clinical features of HCC

The chi-square test revealed that serum miR-424 expression was associated with serum AFP (P=0.048), vein invasion (P=0.006) and TNM stage (P=0.003). However, it was not correlated with gender (P=0.133), age (P=0.076), tumor size (P=0.420), liver cirrhosis (P=0.344), tumor number (P=0.899) and tumor differentiation (P=0.247) (**Table 1**).

Low serum miR-424 was correlated with poor clinical outcome of HCC

The survival analysis showed that the 5-year OS rate of HCC patients in the low serum miR-424 expression group was 28.57%, which was significantly lower than that of patients (45.65%) in the high serum miR-424 expression group (P=0.018) (**Figure 2**).

The 5 year DFS of the HCC patients in the low serum miR-424 expression group and high serum miR-424 expression group were 16.32% and 34.78% respectively. Significant differ-

Table 2. Multivariate analysis of parameters associated with OS and DFS of HCC patients

Parameter	Overall survival			Disease-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
Serum AFP	2.185	0.956-5.362	0.054	1.928	0.912-5.093	0.061
Vein invasion	4.626	1.751-9.845	0.011	4.892	1.944-9.216	0.015
TNM stage	5.320	2.476-13.578	0.007	5.951	2.616-14.983	0.004
Serum miR-204 expression	3.864	1.281-7.950	0.031	4.465	1.579-8.511	0.024

ence regarding to the DFS was detected between the two groups (P=0.008) (**Figure 3**).

Serum miR-424 was an independent risk factor for HCC

Multivariate analysis showed that vein invasion, TNM stage and serum miR-424 expression were independent predictors for OS and DFS of patients with HCC (P<0.05; P<0.01) (**Table 2**).

Discussion

MiR-424 plays an important role in regulation of many important cellular processes such as cell proliferation, migration and differentiation [13-15]. Therefore, deregulation of miR-424 involves in a number of human diseases. The expression level of miR-424 in hair shaft was significantly upregulated only in patients with psoriasis compared with normal controls and those with atopic dermatitis, indicating miR-424 might participate in regulation of psoriasis [16]. Kim et al reported that miR-424 was downregulated in pulmonary arterial hypertension (PAH) and miR-424 might exert its function by regulating apelin and fibroblast growth factor 2 signaling. In addition, reconstitution of miR-424 could ameliorate pulmonary hypertension in animal models [17].

The results of current study revealed that serum miR-424 expression was decreased in HCC patients. In addition, serum miR-424 expression was associated with serum AFP, vein invasion and TNM stage. The HCC patients with lower serum miR-424 had both poorer 5 year OS and DFS. Moreover, serum miR-424 was demonstrated to be an independent predictor of HCC. Consistent with our study, Yu et al showed that down-regulation of miR-424 was found in HCC tissue samples and cell lines. Enforced expression of miR-424 resulted in reduced HCC cell proliferation, migration and invasion *in vitro*, and vice versa. In addition, c-Myb was identified as a direct target of miR-424; indicating miR-424 functioned as a tumor suppressor gene in HCC by downregulating the activity of c-Myb [11]. The expression level of miR-424-5p was significantly down-regulated in anoikis-resistant HCC cells. Ectopic overexpression of miR-424-5p in HCC cells could reverse the resistance to anoikis, suppress EMT process as well as inhibit malignant behaviors. ICAT/CTNNBIP1, which is a potent β-catenin inhibitor, was a direct target of miR-424-5p. Moreover, the clinical data revealed that miR-424-5p was significantly reduced in HCC tissues in comparison with that of the non-cancerous liver tissues. Decreased expression of miR-424-5p was significantly correlated with the progression of this malignant disease, suggesting the potential clinical value of miR-424-5p for HCC [12]. Similarly, Yang et al reported that miR-424 expression level was associated with various important HCC clinical parameters including tumor size, multiple nodules, vein invasion, TNM stage and overall survival; and Akt3/E2F3 axis was demonstrated to be regulated by miR-424 [18].

Similar to HCC, miR-424 functions as a tumor suppressor gene is other types of cancers. Li et al showed that the expression level of miR-424 was reduced in endometrial cancer and inhibition of miR-424 suppressed the growth of endometrial cancer cells by targeting E2F7 [19]. Hershkovitz-Rokah et al reported that the oncogenic BCR-ABL tyrosine kinase fusion gene was a direct target of miR-424 In addition; ectopic expression of miR-424 could not only inhibit proliferation and induce apoptosis of K562 cells, but also sensitize these cells to imatinib treatment; indicating miR-424 might be a promising therapeutic target of chronic myeloid leukemia [20]. Suppressing DNMT-1 increased the expression level of miR-424 and inverse correlations were observed between

DNMT-1 and miR-424 in clinical bladder cancer specimens. The reduction of miR-424 expression was significantly associated with aggressive tumor growth, advanced clinical stage and poor prognosis in bladder cancer [21].

Each single miRNA might have hundreds of downstream targets and its biological functions are closely correlated with the targets. Therefore, it is common to see a specific miRNA might play different roles in different types of cancers or even in the same disease. Wu et al revealed that the expression level of miR-424-5p was significantly up-regulated in pancreatic cancer. In addition, overexpression of miR-424-5p could enhance proliferation, migration and invasion of pancreatic cancer cells, while inhibited cell apoptosis; suggesting that miR-424 might act as an oncogene in pancreatic cancer [22]. MiR-424 was also reported to be up-regulated in colon cancer in different studies, indicating miR-424 might be a positive regulator of colon cancer progression [23, 24].

Taken together, our data demonstrated that serum miR-424 was decreased in patients with HCC. In addition, reduced serum miR-424 expression was associated with advanced clinical stage and poor prognosis of HCC. Therefore, serum miR-424 might be a novel prognostic biomarker for HCC.

Disclosure of conflict of interest

None.

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