Research Article

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Circulating *microRNA-21* as a novel biomarker for hepatocellular carcinoma

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Background & Aims: Several groups have reported the significance of circulating microRNA as a biochemical marker of cancer. To our knowledge, however, there are no reports on the significance of circulating microRNA in hepatocellular carcinoma. The aim of this study was to evaluate the significance of plasma *microRNA-21* level as a biochemical marker for hepatocellular carcinoma.

Methods: Plasma *microRNA-21* level was measured by qRT-PCR in 10 patients before and after curative resection of hepatocellular carcinoma. Plasma *microRNA-21* was also compared in other groups of: 126 patients with hepatocellular carcinoma, 30 patients with chronic hepatitis, and 50 healthy volunteers. The power of *microRNA-21* in differentiating hepatocellular carcinoma from chronic hepatitis or from healthy volunteers was compared to that of α-fetoprotein.

Results: In the 10-patient group, plasma *microRNA-21* levels significantly diminished after surgery compared with the pre-operative values ($p = 0.0125$). Plasma *microRNA-21* level in the 126 patients with hepatocellular carcinoma was significantly higher than in patients with chronic hepatitis and healthy volunteers ($p < 0.0001$, $p < 0.0001$, respectively). ROC analysis of plasma *microRNA-21* yielded an AUC of 0.773 with 61.1% sensitivity and 83.3% specificity when differentiating hepatocellular carcinoma from chronic hepatitis, and an AUC of 0.953 with 87.3% sensitivity and 92.0% specificity when differentiating hepatocellular carcinoma from healthy volunteers. Both sets of values were superior to α-fetoprotein and improved for the combination of *microRNA-21* and α-fetoprotein.

Conclusions: Plasma *microRNA-21* level is a promising biochemical marker for hepatocellular carcinoma.

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Keywords: Hepatocellular carcinoma; microRNA; *microRNA-21*; Plasma; Biomarker.

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Abbreviations: AFP, α-fetoprotein; AUC, area under the receiver–operator characteristic curve; CH, chronic hepatitis; CT, computed tomography; HCC, hepatocellular carcinoma; HV, healthy volunteer; miRNA, microRNA; MRI, magnetic resonance imaging; PIVKA-II, protein induced by vitamin K absence or antagonists-II; qRT-PCR, quantitative RT-polymerase chain reaction; ROC, receiver–operator characteristic; RT, reverse transcription.

Introduction

MicroRNA (miRNA) is a small noncoding RNA gene product known to post-transcriptionally modulate gene expression by negatively regulating the stability or translational efficiency of its target mRNAs [1,2]. MiRNAs control a wide array of biological processes, such as cell differentiation, proliferation, and apoptosis. Aberrant expressions of miRNAs have been widely reported in human cancers with both up- and down-regulation detected in neoplastic cells compared with their normal counterparts [3,4]. Several recent studies reported that miRNAs are stably detectable in plasma and serum [4–6]. Mitchell *et al.* [5] reported that tumor-associated circulating miRNAs are stably detectable in the plasma of human prostate cancer xenograft mouse models and prostate cancer patients, suggesting that their detection could differentiate cancer-bearing individuals from healthy controls. The finding also raised the possibility that assaying miRNAs in plasma or serum may serve as a novel approach for blood-based detection of human cancers. Actually, since the above study, several investigators have reported the significance of some types of plasma miRNAs as biochemical markers for human cancers [7–13].

Hepatocellular carcinoma (HCC) is a common cancer worldwide, especially in Japan and other East Asian countries, and the third most frequent cause of cancer-related deaths in the world [14]. One of the reasons for the high mortality in HCC is that the tumors are frequently detected at a stage when curative resection is no longer feasible because of intrahepatic and extrahepatic metastases. Today, the diagnosis of HCC relies on the finding of a liver mass in radiology imaging studies including ultrasonography, computed tomography (CT), and/or magnetic resonance imaging (MRI). However, the diagnosis of small lesions is relatively inaccurate [15]. One of the common approaches used for screening HCC in a high risk-population is serum tumor markers such as α-fetoprotein (AFP) and protein induced by vitamin K absence or antagonists-II (PIVKA-II). However, the sensitivity and

Table 1. Clinicopathological characteristics of patients with hepatocellular carcinoma (HCC), patients with chronic hepatitis (CH), and healthy volunteers (HVs).

	HCC patients		CH patients	HVs	*p* value	
	(n = 10)‡	(n = 126)	(n = 30)	(n = 50)	(HCC *vs.* CH)	(HCC *vs.* HVs)
Clinical factors						
Gender (male/female)	9/1	99/27	20/10	37/13	0.1683	0.5140
Age (years)*	66 ± 9	63 ± 10	62 ± 8	62 ± 8	0.4062	0.6935
Viral status (B-C-/B+C-/B-C+/B+C+)†	1/3/6/0	14/25/84/3	0/4/26/0		0.1129	
AST (IU/L)*	39 ± 19	38 ± 20	56 ± 28		0.0002	
ALT (IU/L)*	39 ± 20	41 ± 25	57 ± 36		0.0048	
Platelet count (x10^4/μl)*	14.8 ± 5.1	16.1 ± 6.0	15.1 ± 5.8		0.4334	
Prothrombin time (%)*	82 ± 13	76 ± 12	74 ± 11		0.4400	
Albumin (g/dl)*	3.9 ± 0.2	3.9 ± 0.3	3.9 ± 0.4		0.9697	
Total bilirubin (mg/dl)*	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3		0.8564	
Child-Pugh classification (A/B)	8/2	112/14	25/5		0.3693	
Liver cirrhosis (-/+)	6/4	67/59	30/0		<0.0001	
Tumor-related factors						
AFP (ng/ml)*	431 ± 424	8715 ± 46,095	13 ± 16	5 ± 1	0.3039	0.1840
PIVKA-II (mAU/ml)*	736 ± 785	8061 ± 26,319				
Tumor number (single/multiple)	6/4	75/51				
Maximum tumor size (cm)*	3.5 ± 1.8	4.9 ± 3.3				
Vascular invasion (-/+)	8/2	95/31				
TNM staging (I/II/IIIA)	6/2/2	67/16/43				
CLIP scoring (0/1/2/3-)	2/4/3/1	52/37/23/14				
JIS scorings (0/1/2/3-)	1/4/4/1	11/62/27/26				
BCLC staging (A/B/C)	5/3/2	58/37/31				
Edmondson-Steiner grade (I, II/III, IV/unknown)	5/5/0	42/76/8				

*Data are mean ± SD.
†Negative HBs-Ag, positive HBs-Ag, negative anti-HCV Ab, and positive anti-HCV Ab were defined as B−, B+, C−, and C+, respectively.
‡Patients with blood samples before and after surgical resection.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; NL, normal liver; LC, liver cirrhosis; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence; HBs-Ag, hepatitis B surface antigen; anti-HCV Ab, anti-hepatic C virus antibody.

specificity of high serum AFP and PIVKA-II levels for HCC were reported to range from 39–64% and 76–91%, and 41–77% and 72–98%, respectively, suggesting that elevated serum AFP and PIVKA-II levels have insufficient sensitivity and specificity [16–18]. Accordingly, to identify novel biochemical markers for early detection of HCC is desirable.

To our knowledge, there are no reports on the significance of circulating miRNAs in HCC. In this study, we focused on *miRNA-21*, which is one of the first miRNAs detected abundantly in certain human cancers [4,19–21]. *miRNA-21* targets tumor suppressor genes, such as PDCD4, PTEN, and matrix metalloproteinase inhibitors, such as TIMP3 and RECK. Furthermore, *miRNA-21* increased cell proliferation and suppressed apoptosis in a cancer xenograft model, further defining *miRNA-21* as an oncogenic miRNA [22–25]. Overexpression of *miRNA-21* is reported in many types of cancers [26–29]. Also in HCC, it is previously reported that the expression was significantly increased in cancer tissues and cell lines, and that *miRNA-21* contributed to the malignant potential such as cell proliferation, migration, and invasion by reducing the aforementioned targets [30,31]. In other studies, *miRNA-21* was reported to be secreted by cells and detected in plasma [5,32]. It was also confirmed that plasma *miRNA-21* was a useful biomarker for some types of cancer [5,7,9,13]. Thus, we postulated that plasma *miRNA-21* expression could be a novel biochemical marker for HCC. In the present study, we evaluated the usefulness of plasma *miRNA-21* as a biochemical marker for HCC by comparing the expression in patients with HCC and control patients. In addition, we also examined the prognostic significance of plasma *miRNA-21* and investigated the correlation between *miRNA-21* expression in tumoral tissue and its plasma levels.

Materials and methods

Patients and samples

From 10 patients with HCC who had consecutively undergone curative hepatic resection at the Department of Surgery, Osaka University Hospital between January 2010 and February 2010, pre-operative and post-operative plasma samples were collected for the measurement of *miRNA-21*. In the present study, curative resection was defined as complete removal of all macroscopically evident tumors. Post-operative plasma samples were obtained 10–30 days after surgery under the confirmation of no obvious recurrence by ultrasonography, CT, and/or MRI. The clinicopathological features of the 10 patients are shown in Table 1. Plasma

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