Original Article

MicroRNA301 is a potential diagnostic biomarker for hepatocellular cancer

Kun He1,2, Zemin Hu2, Jiahou Ruan2, Qianhong Ma2, Feng Zhong1, Xinsheng Cheng1, Shibo Sun1, Jie Zhou1

1Department of Hepatobiliary Surgery, Nanfang Hospital of Southern Medical University, Guangzhou, China; 2Department of Hepatobiliary Surgery, Zhongshan People’s Hospital, Zhongshan, China

Received February 6, 2015; Accepted April 10, 2015; Epub May 1, 2015; Published May 15, 2015

Abstract: We explored microRNA301 diagnosis value in hepatocellular cancer (HCC), attempting to provide novel insights for early detection, effective prevention, and timely treatment. 42 patients with HCC and 38 controls composed of 9 liver cirrhosis (LC), 9 chronic hepatitis B (CHB) and 20 healthy individuals were investigated in the study. Serum microRNA301 expression levels were detected using fluorescent quantitative polymerase chain reaction (FQ-PCR) technology. ROC curve was performed to evaluate diagnosis value of microRNA301. Meanwhile, the correlations of microRNA301 levels with clinical characteristics were also analyzed. Significantly up-regulated expression of serum microRNA301 was seen in HCC patients compared with the controls (P < 0.05). We also noted that level changes of microRNA301 were associated with differentiation, alpha fetoprotein (AFP), portal vein-emboli and HasAg (P < 0.05), rather than age, gender and tumor size. Based on the area under ROC curve of 0.880, the critical value of microRNA301 was 2.3530 and the sensitivity and specificity were 88.1% and 70.3%, respectively. The results of this study revealed that microRNA301 might function as a potential diagnostic biomarker for HCC.

Keywords: Hepatocellular cancer, MicroRNA301, diagnosis

Introduction

With the development of science technology, cancer diagnosis has been improved in recent years. Such an improvement, however, is not seen in hepatocellular cancer (HCC), one of the most common malignant cancer worldwide with a high incidence and mortality rate in Asia and Africa [1]. The vast majority of HCC patients have been in the advanced stage at the time of diagnosis. The delayed diagnosis makes it impossible to carry out resection surgery, thus results in high death rate of HCC patients [1-3]. At present, HCC screening is performed by alpha fetoprotein (AFP) measurement. Recently, researchers have found that level of microRNAs was associated with the development of HCC [4-6]. It is therefore of great significance to evaluate the diagnosis value of microRNAs in HCC.

Previous studies reported that microRNAs, a class of non-coding small RNAs, show certain concentration in serum [7]. MicroRNAs mostly bind to target genes and then regulate their expressions by inhibiting or interrupting translation. It has been demonstrated that microRNAs involve in an array of physiological and pathological processes, including organism development, inflammation response and tumorigenesis. Aberrant expression of microRNAs are associated with initiation and progression of HCC [8], which indicates that microRNAs might act as non-invasive biomarkers to detect HCC.

MicroRNA301 is a SKA2 intron-located microRNA, up-regulation of which stimulates clonogenicity, invasion, and microvessel density, while its down-regulation suppresses proliferation, migration, and tumor growth [9]. Moreover, microRNA301 could regulate immunity and proteome diversity during the self-renewal of human pluripotent stem cells by targeting SFRS2 and MDB2a [10]. Recent researches showed that over-expressed microRNA301 was found in prostate cancer cell and pancreatic cancer tissues [11, 12], while significantly decreased level of microRNA301 was observed in the cholangiocarcinoma cells [13]. Additionally, expression of microRNA301 in HCC
MicroRNA301, potential biomarker for HCC

**Materials and methods**

**Study population**

42 patients diagnosed as HCC were recruited from Zhongshan People’s Hospital. Individuals previously received any anti-HCC treatments were excluded from the study. 38 controls containing 20 healthy individuals, 9 liver cirrhosis (LC) patients and 9 chronic hepatitis B (CHB) patients were enrolled during the same period of time. The diagnosis of HCC, LC and CHB was performed by two pathologists independently based on morphology, molecular and immunophenotype detection, complying with the classification standard of World Health Organization. Informed consent was obtained from each participant. The study was approved by Ethical Review Committee of the hospital.

**RNA extraction**

Peripheral blood was obtained from each participant. RNA was isolated from 200 µl fresh serum using miRNeasy mini kit (Tiangen, Beijing city, China) following the manufacturer’s proposals. Then RNA was exposed in 200 µl Rnase-free water and stored at -80°C until utilized. The quality and purity of total RNA were examined via 260 nm of optical density survey using a NanoDrop ND-1000 spectrophotometer (USA). cDNA was synthesized using miScript Reverse Transcription Kit (Qiagen, Germany).

**Fluorescent quantitative PCR (FQ-PCR) assay**

MicroRNA301 level was measured by FQ-PCR technology after cDNA reverse transcription. The performance was conducted according to the instructions of miRcute miRNA detection kit (Tiangen, Beijing city, China). MicroRNA301 expression level was normalized by GAPDH control. All experiments were operated in triplicate. The quantity of microRNA301 was calculated by the $2^{-\Delta\Delta C_T}$ method.

**Statistical analyses**

SPSS 20.0 was performed for all statistical data analyses. $P < 0.05$ was considered to be statistically significant. Differences in relative expression level of microRNA301 among the groups were performed using the one-way ANOVA analysis. ROC (receiver operating characteristic) curve was used to evaluate the diagnostic value of serum microRNA301.

**Results**

**Increased level of microRNA301 in HCC patients**

FQ-PCR assay was employed to measure serum microRNA301 expression for each participant. We found that the expression level of microRNA301 was significantly elevated in patients with HCC compared to the controls ($P < 0.05$) (Figure 1). This outcome implicated that microRNA301 might play a role in the pathogenesis of HCC.

**Correlation between microRNA301 expression level and clinical-pathological characteristics**

To examine if microRNA301 concentration was associated with the clinical parameters, we classified the patients by gender, age, tumor size, differentiation, portal vein-emboli, AFP and HasAg. As shown in Table 1, serum microRNA301 level was associated with multiple parameters including differentiation, portal vein-emboli, AFP and HasAg ($P < 0.05$), whereas no significant correlation was found for age, gender and tumor size.
Table 1. Associations of serum microRNA301 expression with clinical indicators of HCC patients

<table>
<thead>
<tr>
<th>Pathological parameters</th>
<th>microRNA301-high expression n=36 (%)</th>
<th>microRNA301-low expression n=6 (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (86.67)</td>
<td>4 (13.33)</td>
<td>0.780</td>
</tr>
<tr>
<td>Female</td>
<td>12 (83.33)</td>
<td>2 (16.67)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 50</td>
<td>16 (87.50)</td>
<td>2 (12.50)</td>
<td>0.795</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>26 (84.62)</td>
<td>4 (15.38)</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>0.554</td>
</tr>
<tr>
<td>≤ 5 cm</td>
<td>10 (80.00)</td>
<td>2 (20.00)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>32 (87.50)</td>
<td>4 (12.50)</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td>0.041</td>
</tr>
<tr>
<td>Mod-well</td>
<td>13 (69.23)</td>
<td>4 (30.77)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>29 (93.07)</td>
<td>2 (6.90)</td>
<td></td>
</tr>
<tr>
<td>Portal vein-emboli</td>
<td></td>
<td></td>
<td>0.021</td>
</tr>
<tr>
<td>Positive</td>
<td>17 (70.59)</td>
<td>5 (29.41)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>25 (96.00)</td>
<td>1 (4.00)</td>
<td></td>
</tr>
<tr>
<td>AFP</td>
<td></td>
<td></td>
<td>0.014</td>
</tr>
<tr>
<td>&lt; 400 μg/L</td>
<td>16 (68.75)</td>
<td>5 (31.25)</td>
<td></td>
</tr>
<tr>
<td>&gt; 400 μg/L</td>
<td>26 (96.15)</td>
<td>1 (3.85)</td>
<td></td>
</tr>
<tr>
<td>HasAg</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (61.54)</td>
<td>5 (38.46)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29 (96.55)</td>
<td>1 (3.45)</td>
<td></td>
</tr>
</tbody>
</table>

Detection value of serum microRNA301 in HCC diagnosis

ROC analysis was performed to determine the diagnostic significance of microRNA301 for HCC. The area under the curve was 0.880. The critical value was 2.3530 with 88.1% diagnostic sensitivity and 70.3% specificity, suggesting microRNA301 might serve as an essential biomarker for HCC (Figure 2).

Discussion

HCC, one of the most common cause of death from malignancy worldwide, caused by a variety of etiologies including LC, chronic hepatitis, or immune-related causes [15-19]. So LC and CHB patients were selected as control group together with the normal individuals. Recently, researches have identified a number of genetic alterations related with the development of HCC [20, 21]. At the same time, pathologic studies found some important molecules and pathways involved in hepato-carcinogenesis, including microRNAs [22-27].

MicroRNAs, which are endogenously expressed, play important roles in modulating cells growth, differentiation, apoptosis, and tumorigenesis [28-31]. It is believed that level changes of microRNAs result in diverse types of human malignancy [32-35], thus detection of these cancer-related microRNAs contributes to early diagnosis and prevention. Until now, the expression pattern of microRNA301 in HCC patients was still unclear.

We tested serum microRNA301 level in the present study and obtained the evidence that microRNA301 expression was significantly increased in the serum of HCC patients. Such phenomenon was also previously observed in prostate cancer, pancreatic cancer, hepatocellular carcinomas and oral cancer [11, 12, 14, 36]. However, this result seemed to contradict...
some studies, which demonstrated a clear down-regulation of microRNA301 in cholangiocarcinoma, HCV-infected human hepatoma cells and small cell lung cancer [13, 37, 38]. The possible explanation might be that microRNA301 functions differentially in different cancers with distinct etiologies. Meanwhile, the experiment conditions of each study was also related to the divergence. To simplify detection procedure, we selected patients’ serum to perform measurements [39]. As far as we are aware, this research was the first study evaluating the diagnosis value of serum microRNA301 for HCC.

Besides, we also demonstrated significant relationship between microRNA301 levels and clinical characteristics including differentiation, portal vein-emboli, AFP and HasAg. Therefore, the aforementioned parameters rather than gender, age and tumor size, were confounding factors for microRNA301 expression, indicating the clinical significance of microRNA301 in identifying the patients with HCC. MicroRNA301 diagnosis value for HCC was tested using ROC curve analysis. Our results revealed bigger areas, and higher sensitivity and specificity. That means, microRNA301 provided diagnosis value for HCC, approaching accurate diagnostic results, higher true positive rate and true negative rate. Therefore, serum microRNA301 can be used as an effective biomarker in HCC diagnosis.

In summary, the study had shown some evidence that serum level of microRNA301 was significantly up regulated in HCC patients, and microRNA301 could be used as a biomarker to detect HCC. The result of our study might provide a valuable strategy for HCC diagnosis. Further clinical significance remains to be verified in well-designed studies including a large number of participants.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jie Zhou, Department of Hepatobiliary Surgery, Nanfang Hospital of Southern Medical University, Guangzhou, China. E-mail: jiezhouji@126.com

References


5606


