Down-regulation of miR-655-3p predicts worse clinical outcome in patients suffering from hepatocellular carcinoma

X.-Q. ZHAO, B. LIANG, K. JIANG, H.-Y. ZHANG

Hospital & Institute of Hepatobiliary Surgery, Chinese PLA General Hospital, Beijing, China

Xiang-qian Zhao and Bin Liang contributed equally to this work

Abstract. – OBJECTIVE: MiR-655-3p has been reported to play important roles in tumor initiation, development, and metastasis in several cancers. This study aimed to assess the potential role of miR-655-3p in the pathogenesis of hepatocellular carcinoma (HCC).

PATIENTS AND METHODS: The expression levels of miR-655-3p in HCC tissues were detected by qPCR. The relationship between clinicopathologic characteristics and miR-655-3p was analyzed by chi-square test. Kaplan-Meier curves and multivariate Cox proportional models were used to study the impact on clinical outcome.

RESULTS: miR-655-3p was significantly down-regulated in HCC tissues compared to normal adjacent liver tissues ($p < 0.01$). Low miR-655-3p expression was observed to be closely correlated with positive microvascular invasion, advanced tumor stage and lymph node metastasis ($p < 0.05$, respectively). The results of Kaplan–Meier analysis showed that patients with high miR-655-3p expression lived shorter than those with low miR-655-3p expression (Log-rank test, $p = 0.0002$). Multivariate analysis revealed that miR-655-3p was an independent risk factor for HCC (HR=1.533, 95% CI: 0.988-3.891; $p = 0.002$).

CONCLUSIONS: Our data showed that low expression of miR-655-3p was associated with significant characteristics of patients with HCC, and it could function as a potential unfavorable prognostic biomarker.

Key Words: MiR-655-3p, Hepatocellular carcinoma, Prognosis.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third most common cause of cancer death globally. Despite the recent advances in clinical and experimental oncology, the prognosis of HCC still remains poor. Notably, most patients diagnosed with advanced-stage HCC die of recurrence and/or metastasis. Thus, it is important to identify new markers to predict more accurately prognosis of an individual patient.

MicroRNAs (miRNAs) are single-stranded, small noncoding RNAs of 18-22 nucleotides in length, first discovered in the early 1990s in Caenorhabditis elegans. It has been known to us that miRNAs regulate the expression of various genes at the post-transcriptional level by binding to the 3′-untranslated region (3′-UTR) of their target mRNAs. Due to their widespread modulation on protein-coding genes, miRNAs have various physiological and pathological functions. Numerous studies have demonstrated that miRNAs can function as oncogenes or tumor suppressors. Currently, some studies revealed that changes in expression of miRNAs may be used as robust biomarkers for cancer risk, diagnosis, and prognosis. Zhang et al. showed that low miR-613 expression was associated with lower progression-free and overall survival. Chen et al. found that serum miR-182 and miR-331-3p were associated with postoperative survival of HCC patients, and they further identified them to be independent prognostic factors for patients with HCC. A previous paper reported that miR-655-3p was significantly down-regulated in HCC tissues and HCC cell lines, and overexpression of miR-655-3p suppressed cell proliferation and migration in HCC by directly targeting ADAM10. However, the clinical significance of miR-655-3p was never reported in HCC.

In the present work, we performed RT-PCR to determine the expression levels of miR-655-3p. Subsequently, we analyzed the association between the miR-655-3p expression and various clinicopathological factors of HCC patients. Fi-
Down-regulation of miR-655-3p in hepatocellular carcinoma

nally, we further studied the correlation between miR-655-3p expression and prognostic value.

Patients and Methods

Patients and Tissue Samples

HCC tissue slice samples were obtained from 188 patients diagnosed with HCC who had undergone a curative hepatectomy in the Chinese PLA General Hospital. After resection, frozen HCC tissues were collected immediately after resection. All the diagnosis was pathologically confirmed. None of the patients had undergone preoperative intervention therapy or chemotherapy. The clinicopathological information of the patients is summarized in Table I. Written informed consent for the biological studies was obtained from each patient involved in the study. The study was approved by the Ethical Review Committee of the Institute. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration.

RNA Isolation and Quantitative Real-Time PCR

Total RNA from tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) for mRNA analyses. cDNA was synthesized with the ReverTra Ace®qPCR RT Kit (FSQ-101; Toyo-bo, Osaka, Japan). Real-time PCR was used to determine the expression of miR-655-3p in HCC tissues. Quantitative real-time RT-PCR was performed by using the SYBR green reagent with an ABI Prism 7000HT sequence detection system. Primers for miR-655-3p and the internal control GAPDH gene were purchased from Ambion (Applied Biosystems, Foster City, CA, USA). Relative quantification of miR-655-3p expression was calculated using the $2^{-ΔΔCT}$ method. All experiments were repeated three times.

Statistical Analysis

Data are presented as the mean ± s.d. from at least three independent experiments. The data were assessed using Graphpad Prism 5.0 software (Graphpad, La Jolla, CA, USA) and SPSS 20.0 software (IBM, New York, NY, USA). Comparisons between groups for statistical significance were performed with a two-tailed paired Student’s t-test. Correlations between clinical characteristics and miR-655-3p expression were evaluated using the Chi-squared test. Kaplan-Meier analysis and the log-rank test were performed to identify survival differences in HCC patients. Prognostic significance of each variable to overall survival was analyzed using the Cox regression model. $p < 0.05$ was used to indicate a statistically significant difference.

Results

miR-655-3p Expression is Decreased in HCC Tissues

To determine whether miR-655-3p was aberrantly expressed in HCC tissues, qRT-PCR was performed to detect the expression levels of 188 paired clinical HCC tissues and adjacent normal tissues. Our results showed that the average expression level of miR-655-3p was significantly lower in HCC than that in matched normal tissues (Figure 1). These data suggested that miR-655-3p may play a negative role in the progression of miR-655-3p.

Association Between Clinicopathological Characteristics and miR-655-3p Expression

To further establish the correlation of miR-655-3p expression with clinical prognosis, we manually divided the HCC patients into two groups (high group and low group) according to the average expression of miR-655-3p. The correlations between miR-655-3p expression and the clinicopathological characteristics of the patients are presented in Table I. The results showed that

Figure 1. qRT-PCR analysis of miR-655-3p expression in HCC tissues and matched adjacent normal tissues. miR-655-3p was significantly downregulated in HCC tissues.
miR-655-3p expression was significantly related to microvascular invasion \( (p = 0.000) \), tumor stage \( (p = 0.006) \), and lymph node metastasis \( (p = 0.001) \). However, there were no significant correlations between miR-655-3p expression and other clinicopathologic features, such as age, HBsAg status, or tumor size. Our findings supported the notion that miR-655-3p down-regulation may be associated with tumor progression.

**Down-Regulation of miR-655-3p Confers Poor Prognosis in HCC Patients**

To further analyze the significance of miR-655-3p in terms of clinical prognosis, we performed the log-rank test and the results showed that the overall survival of HCC patients with low miR-655-3p expression was significantly shorter than those with high miR-655-3p expression \( (p = 0.0002; \text{Figure 2}) \). In the univariate analysis, microvascular invasion \( (HR = 2.451, 95\% \text{ CI}: 1.113-4.583, p = 0.006) \), tumor stage \( (HR = 3.315, 95\% \text{ CI}: 1.776-6.451, p = 0.003) \), lymph node metastasis \( (HR = 1.771, 95\% \text{ CI}: 1.021-3.668, p = 0.002) \), and expression of miR-655-3p \( (HR = 1.628, 95\% \text{ CI}: 1.144-4.337, p = 0.001) \) were associated with poor survival. Further multivariate COX regression analysis confirmed that miR-655-3p expression functioned as predictors of poor prognosis \( (HR=1.533, \text{CI}: 0.988-3.891, p = 0.002) \), shown in Table II.

Table I. Association between miR-655-3p expression and clinicopathological parameters of HCC.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>miR-655-3p</th>
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<tbody>
<tr>
<td></td>
<td>Low ((n = 96))</td>
</tr>
<tr>
<td>Sex</td>
<td>Male: 72 32 40 Male: 116 64 52</td>
</tr>
<tr>
<td></td>
<td>Female: 116 64 52 Female: 69 39 30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>(\geq 60): 119 57 62 (\leq 60): 69 39 30</td>
</tr>
<tr>
<td>HBsAg status</td>
<td>Positive: 90 49 41 Negative: 98 47 51</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>(\geq 5): 109 61 48 (&lt; 5): 79 35 44</td>
</tr>
<tr>
<td>Tumor nodes</td>
<td>Multi: 87 44 43 Single: 101 52 49</td>
</tr>
<tr>
<td>Serum AFP (ng/dl)</td>
<td>(\geq 200): 104 54 50 (&lt; 200): 84 42 42</td>
</tr>
<tr>
<td>Microvascular invasion</td>
<td>Yes: 109 68 41 No: 79 28 51</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>I+II: 89 36 53 II+IV: 99 60 39</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>No: 98 39 59 Yes: 90 57 33</td>
</tr>
</tbody>
</table>

Table II. Univariate and multivariate analyses of prognostic factors in HCC patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>HR 95% CI (p)</td>
<td>HR 95% CI (p)</td>
</tr>
<tr>
<td>Sex</td>
<td>1.334 (0.615-1.931)</td>
<td>1.033 (0.514-1.673)</td>
</tr>
<tr>
<td>Age</td>
<td>1.517 (0.933-2.661)</td>
<td>1.316 (0.732-2.016)</td>
</tr>
<tr>
<td>HBsAg status</td>
<td>2.416 (1.533-2.991)</td>
<td>1.933 (1.237-2.144)</td>
</tr>
<tr>
<td>Tumor size</td>
<td>2.118 (1.347-3.351)</td>
<td>1.892 (1.231-3.033)</td>
</tr>
<tr>
<td>Tumor nodes</td>
<td>2.655 (1.235-3.884)</td>
<td>2.135 (1.144-2.335)</td>
</tr>
<tr>
<td>Serum AFP</td>
<td>2.655 (1.235-3.884)</td>
<td>2.135 (1.144-2.335)</td>
</tr>
<tr>
<td>Microvascular invasion</td>
<td>2.451 (1.113-4.583)</td>
<td>2.015 (0.933-3.114)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>3.315 (1.776-6.451)</td>
<td>2.674 (1.993-5.113)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>1.771 (1.021-3.668)</td>
<td>1.417 (1.432-4.166)</td>
</tr>
<tr>
<td>miR-655-3p</td>
<td>1.628 (1.144-4.337)</td>
<td>1.533 (0.988-3.891)</td>
</tr>
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</table>

Figure 2. Kaplan-Meier curves of survival in patients with HCC.
in Table II). Taken together, miR-655-3p expression was an independent predictor for overall survival.

Discussion

HCC is one of the most common and aggressive solid organ tumors in many countries. Although the therapeutic approach for HCC has changed significantly in the past decade, the prognosis of HCC patients remains poor. Investigating new therapeutic modalities and identifying prognostic biomarkers for HCC may help improve the therapy methods. Recently, more and more evidence showed that miRNAs served as a tumor promoter or tumor suppressor in all types of cancer by regulating their targeting genes. Shao et al. reported that elevated expression of miR-519a was observed in HCC tissues, and overexpression of miR-519a promotes proliferation and inhibits apoptosis of hepatocellular carcinoma cells by targeting FOXF2. Huang et al. showed that miR-663a distinctly inhibited HCC cell proliferation, migration and invasion by targeting HMGA2. Zheng et al. revealed that ectopic expression of miR-216b produced a suppressive effect on the growth of HCC cells by targeting Forkhead box protein M1. Those findings provide the support that miRNAs may be used to predict the prognosis of tumor patients.

Several researches have reported that miR-655-3p function as a tumor suppressor in some tumors. For instance, Wang et al. found that miR-23b is highly downregulated in human esophageal squamous cell carcinoma and its overexpression suppresses cell invasion by targeting pituitary tumor-transforming gene-1. Lv et al. showed that miR-663a distinctly inhibited HCC cell proliferation, migration and invasion by targeting HMGA2. Zheng et al. revealed that ectopic expression of miR-216b produced a suppressive effect on the growth of HCC cells by targeting Forkhead box protein M1. Those findings provide the support that miRNAs may be used to predict the prognosis of tumor patients.

In the present work, we performed the RT-PCR and found that the expression of miR-655-3p in HCC specimens was lower than adjacent normal tissues. We also found that the downregulation of miR-655-3p may be markedly associated with the advanced tumor progression, such as tumor stage and microvascular invasion, and lymph node metastasis. Furthermore, Kaplan-Meier analysis with the log-rank test showed that patients with high miR-655-3p expression lived shorter than those with low miR-655-3p expression. More importantly, Multivariable Cox proportional hazards regression analysis confirmed that HULC low expression was an independent prognostic factor in patients with HCC.

Conclusions

Our data give preliminary evidence that miR-655-3p might have potential value for predicting poor prognosis in HCC patients. However, the current paper has not elucidated the exact molecular mechanisms of miR-655-3p in HCC. Further investigation was needed.

Conflict of interest

The authors declare no conflicts of interest.

References


