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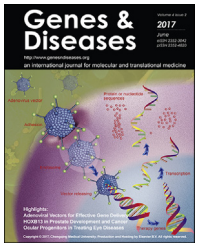

FULL LENGTH ARTICLE

The potential value of microRNA-4463 in the prognosis evaluation in hepatocellular carcinoma☆

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Abstract The purpose of this study is to measure the expression of microRNA-4463 and microRNA-6087 between normal persons and patients with hepatocellular carcinoma (HCC), and to clarify the meaning of them in the prognosis evaluation in HCC. Forty-five samples from healthy people and patients, who had been diagnosed with hepatocellular carcinoma before any treatment, were collected to study respectively. Real-time PCR was used to detect the expression of miRNA-4463 and miRNA-6087 in the serum of control group and hepatocellular carcinoma patients. The expression of miR-4463 in the serum of HCC patients was significantly higher than that in control group ($P < 0.05$), and the expression level was independent of gender, tumor size, cell types, stages, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) and HBsAg status ($P > 0.05$). But there was a significant difference of different level of AFP in HCC ($P < 0.05$), and the difference between the group of AFP lower than 400 ug/l and the control group is statistically significant ($P < 0.05$). Besides, the survival time had showed a significant difference at the high and low expression levels

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($P < 0.05$). But the expression level of miRNA-6087 was no difference in HCC and control group. The disorder of miRNA-4463 occurred in HCC, even the AFP level doesn't rises. What's more, patients who get the high level of miRNA-4463 seem to have a shorter survival time. And it contributes great to the prognostic evaluation. This is the first study to illustrate the potential significance of miRNA-4463 in the prognosis in HCC.
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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the worldwide, and lays on the third leading cancer-related deaths,[1–3] coming with the epidemiological characteristics of high degree of malignancy, high metastasis rate, high recurrence rate and low survival rate.[4] HCC is a huge threat for human's lives and healthy, and becomes a huge challenge for the maintenance of human public health.[5–7] Hepatocellular carcinoma is the imbalance in the regulation of gene transcription level, which leads to the formation of clonal abnormal proliferation of new organisms. The etiopathology of HCC is complicated such as the activation of many oncogenes, and the inactivation of tumor suppressor genes.[8] Because of the lack of effective means of early diagnosis, most patients were diagnosed in the advanced stage with distant metastases or intrahepatic metastasis. In spite of given aggressive treatment, such as liver transplantation, molecular biological treatment, the five-year survival rate is within 30%.[9] Because of the poor prognosis, and the short survival time, early detection and diagnosis are of great at significance. And early diagnosis could help to receive timely effective treatment, finally to improve the prognosis and prolong the survival period.

MicroRNA (miRNA) is a single stranded, endogenous non-coding small molecule RNA.[10] A number of studies have confirmed that miRNAs may play an important role in the diagnosis and targeted therapy of cancer.[11–13] The abnormal expression of some specific miRNAs had been demonstrated in specimens of liver.[14,15] Studies have shown that many miRNAs were participated in the multiplication and metastasis of HCC, which having important significance for the treatment and the prognosis. This study means to make clear that the relationship of the expression level of miR-4463 with pathology and physiology of HCC. And we had tried to analysis the correlation between the expression of miR-4463 and the clinical characteristics. But there was no related research of the expression of miR-4463 in HCC.

Materials and methods

Case group and control group

Forty-five samples with HCC were collected from First Affiliated Hospital and Nanhua Hospital of University of South China, without any treatment. All patients were diagnosed by two methods of ultrasound, CT and histopathology, which combined with related-history and the level of serum AFP.[16] Forty-five normal samples were obtained from the physical examination center in Nanhua Hospital of University of South China. All participators had written informed consent before the study, which was permitted by the Ethics Committee of University of South China. 2 ml peripheral blood was obtained from all the patients and volunteers. Then the upper serum were obtained after centrifugation and stored in −80 °C.

The HCC group includes 12 females and 33 males (age 31−80 years, mean 57.64 ± 11.52 years). And the control group was made up of 18 females and 27 males (aged 28−72 years, mean 45.22 ± 11.28 years).

RNA extraction

200 ul serum and 1000 ul QIAzol Lysis Reagent were mixed in tube. Then 7 ul miRNeasy Serum/Plasma Spike-In Control (Qiagen, Germany) was added and mixed thoroughly. 200 ul chloroform was added to the tube and shook vigorously for 15 s and incubated for 2 min, which followed by centrifugation for 15 min at 12000× *g* at 4 °C. Then 600 ul of the upper aqueous phase was transferred to a new collection tube, and 900 ul ethanol was put into the tube and mixed thoroughly. Each 700 ul of the mixture was added into an RNeasy MinElute spin column which with a 2 ml collection tube, and centrifuged. Then 700 ul Buffer RWT was put into the RNeasy MinElute spin column, and centrifuged. Then 500 ul Buffer RPE was added into the RNeasy MinElute spin column, and centrifuged. 500 ul of 80% ethanol was put into the RNeasy MinElute spin column, and centrifuged. A new 2 ml collection tube was placed into the RNeasy MinElute spin column, and centrifuged, as to dry the spin column membrane. A new 1.5 ml collection tube was placed into the RNeasy MinElute spin column. Then 14 ul RNase-free water was added directly to the center of the spin column membrane, and centrifuged. The leftover was the total RNA.

cDNA synthesis

The reagents of RNA reverse transcription to cDNA had used miScript Reverse Transcriptase Kit (Qiagen, Germany). We had mixed the 3 ul purified RNA, 4 ul of 5× miScript HISpec Buffer, 2 ul of 10× miScript Nucleics Mix, 9 ul of RNase-free water and 2 ul of miScript Reverse Transcriptase Mix. In brief, the final volume in the tube was 20 ul. Incubated for 60 min at 37 °C, 5 min at 95 °C and 30 min at 4 °C. Finally 200 ul RNase-free water had been added into the tube, and stored the whole at −20 °C.

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