Research Article

Expression of microRNA-132 in patient with liver cancer and mediation of microRNA-132 on cell growth of liver cancer cell

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Abstract

We detected the expression of microRNA-132 in Chinese patient with liver cancer and evaluated down-regulation of microRNA-132 affect on cell growth and apoptotic of liver cancer cell. Twenty four patients with liver cancer and 24 normal patients were obtained from the 105th hospital of People's Liberation Army. We measured the expression of microRNA-132 in all selected patients using quantitative RTPCR (qRT-PCR). Lastly, cell growth and apoptotic of liver cancer Human liver cancer MHCC97H cells after anti-microRNA-132 transfection were evaluated using CCK-8 solution and annexin V-FITC and propidium iodide kit. There was significant suppression in microRNA-132 expression of exosome and whose liver cancer cell, comparison with normal patients. Down-regulation of microRNA-132 could inhibit cell growth and activated apoptotic of liver cancer cell. These findings indicate that microRNA-132 provides increased sensitivity of detection than normal patients. MicroRNA-132 may serve as a potential biomarker for treatment on liver cancer.

Introduction

Liver cancer is one of most common cancers (Wolosz et al., 2014). Every year, about 64 thousands of people all over the world are initially diagnosed with liver cancer, among which more than half are Chinese (Zhang et al., 2014b). In China, the leading cause of liver cancer's remarkable rise lies in the evolution of liver cirrhosis after infection of HBV (Allam et al., 2010). Data on 1990s showed that fatality rates of liver cancer are ranked the second (Tang, 2001). Liver cancer is progressing fast and its attack is concealed (Outwater, 2010). Consequently, intra-hepatic or extra-hepatic metastasis has occurred for most patients at initial diagnosis, thus they lost chances to receive radical treatment (Behe et al., 2003). For a few patients, disease can be found at early stage and diagnosis as well as treatment can be conducted, thus they can live longer (Xie et al., 2015).

Pharmacogenetics is a subject studying effects of genetic polymorphism on drug reaction (Gonzalez-Vacarezza et al., 2013). In view of functional genomics and molecular pharmacology, pharmacogenomics is developed on the basis of pharmacogenetics at the end of 1990s and is an achievement of implementation of HGP (Vasku et al., 2009). Its main focus is to study pharmacogenetics by using results of genomics (Li et al., 2013). More specifically, it is to study the hereditary basis of polymorphism of drug reactions, to prove and explain modes of action as well as toxic and adverse effects of drug therapeutic effects and actions from the perspective of molecular level, which provides a platform to develop new drugs and guide rational drug use (Lam et al., 2013). Therefore, security and effectiveness of drug use are increased and adverse reactions are avoided. Meanwhile, costs and risks of drug therapy decrease (Kim et al., 2013).

MicroRNA is a non-coding single stranded RNA with a length of about 22 nucleotides, which is formed by Dicer enzyme-processing of precursors of single stranded RNA in cell nucleus and cytoplasm. It was recognized early in 1993 (Zhang et al., 2014a). It is single structured and participates in essential biological functions such as cell development, apoptosis and metabolism, etc (Wang et al., 2013). As the important role of microRNA in some tumors has been confirmed, many scholars conducted initial studies on relationship between microRNA and occurrence, development and metastasis of liver cancer (Toffanin et al., 2011; Wang et al., 2014). Results showed that microRNA was closely related with liver cancer.

Materials and Methods

Participants

Twenty four patients with liver cancer and 24 patients other than liver cancer were obtained from the 105th Hospital of People's Liberation Army. Blood samples of all patients were gathered and stored at -80°C for analysis. The tumor type and the grade of liver cancer were diagnosed based on the criteria of World Health Organization (WHO) and International Union Against Cancer (UICC) TNM classification.

Serum samples

Firstly, peripheral blood from all participants was gathered and centrifuged at 2,000 rpm for 10 min at 4°C. Then, the supernatants were gathered and centrifuged at 12,000 xg for 10 min at 4°C. The serum samples were stored at −80°C for analysis. Total exosome isolation reagent was used to isolate exosomes from serum samples following the manufacturer's protocol (Invitrogen). 0.2 mL of total exosome isolation reagent (Invitrogen) and 1 mL of serum were blended and incubated for 30 min at 4°C. Then, exosomes was gathered and centrifuged at 10,000 ×g for 10 min at room temperature.

Quantification of serum microRNA-132

Total RNA was isolated using isothiocyanate-phenol/chloroform extraction procedures. SYBR Premix Dimer Eraser kit (TaKaRa, Shiga, Japan) was used to perform real-time quantitative RTPCR (qRT-PCR) using ABI Prism 7900HT Detection System (Applied Biosystems, Foster City, CA).

Anti-microRNA-132 plasmid transfection

Anti-microRNA-132 plasmid: 5'-CGCCAAUAUU-UACGUGCUGCUA-3', the negative control: 5'-CAGUACU-UUUGUGUAG UACAA-3'. Anti-microRNA-132 plasmid and negative control plasmid were structured by Sangon Biotech (Shanghai, China) and transfected into U87 cell using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions.

Cell culture and cell viability

Human liver cancer MHCC97H cells were obtained from The First Hospital of Anhui Medical University and were cultured in DMEM culture medium (Gibco, Billings, MT, USA) containing 10% fetal blood serum (HyClone-Thermo Scientific, Germany) supplemented with 100 U of penicillin/mL, 100 mg of streptomycin/mL at 37°C under a humidified atmosphere of 5% CO_2. MHCC97H cells-transfected was seeded onto 96-well-plate (1×10^3 cell/well). Then, 10 µL of thawed CCK-8 solution (Wuhan Boshide Biotechnology Co., Ltd, Wuhan, China) was added to MHCC97H cells-transfected and incubated for 4 hours at 37°C. Cell viability was read using the microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm/600 nm.

Cell apoptotic

MHCC97H cells-transfected were analyzed by flow cytometry (Beckman Coulter Inc., Miami, FL, USA) using the annexin V-FITC/propidium iodide Apoptosis Detection kit according to the manufacturer's instructions (BestBio Biotechnology Co., Ltd, China). MHCC-97H cells-transfected was seeded onto 6-well-plate ($1-2 \times 10^6$ cell/well) and washed by ice PBS. MHCC97H cells was gathered and cultured with annexin V-FITC and propidium iodide on ice for 30 min in the dark. Cell apoptotic were measured using flow cytometry (Beckman Coulter Inc., Miami, FL, USA).

Statistical analysis

Data were expressed as the means ± standard error (S.E.M.) and Kruskal-Wallis test were performed to determine the significance of the differences. A value of <0.05 was considered significant.

Results

Characterization of participants

There was no significant in age, sex, HBV status, liver cirrhosis and TNM staging in liver cancer and normal patients (Table I).

Serum microRNA-132 expression in higher liver cancer patients

In this study, there was no significant difference in age (≥ 60 or < 60 years), sex and HBsAg of low microRNA-132 expression and high microRNA-132 expression (Table II). However, cases of liver cirrhosis of low microRNA-132 expression was higher than that of high microRNA-132 expression. The statistical analysis revealed that high level of serum microRNA-132 expression positively correlated with TNM staging.

Correlations of microRNA-132 expression and patient clinicopathologic characteristics

To determine the correlations of microRNA-132 expre-

Table I		
Characterization of participants		
	Patient without liver cancer (n = 24)	Liver cancer group (n = 24)
Age (years)	62.2 ± 6.9	63.1 ± 7.2
Sex		
Female	9	9
Male	15	15
HBV status		
Positive	0	20
Negative	24	4
Liver cirrhosis		
Positive	0	19
Negative	30	5
TNM staging		
I		7
II		6
III-IV		11

ssion and patient clinicopathologic characteristics, microRNA-132 expression was measured by qRT-PCR. There was significant suppression in microRNA-132 expression of exosome and whose liver cancer cell, comparison with control group (Figure 1). Meanwhile, microRNA-132 expression of exosome cell was higher than that of whose cell in liver cancer cell or normal control group.

Mediation of microRNA-132 on cell growth of liver cancer cell

To determine whether down-regulation of microRNA-132 played a role in the cell growth of liver cancer cell, we transfected MHCC97H cells with anti-microRNA-132. As shown in Figure 2, transfection of anti-microRNA-132 inhibited cell growth of liver cancer cell, comparison with control group.

Mediation of microRNA-132 on apoptotic of liver cancer cell

We have showed down-regulation of microRNA-132 affect on apoptotic of liver cancer cell. These results indicated down-regulation of microRNA-132 activated apoptotic of liver cancer cell, comparison with control group (Figure 3).

Discussion

In our study, cases of liver cirrhosis of low microRNA-132 expression were higher than that of high microRNA-132 expression. The statistical analysis revealed that high level of serum microRNA-132 expression positively correlated with TNM staging.

MiR-132 is highly conserved in evolution and possesses same arrays and structures in species like human beings, rats, mice and apes (Guo et al., 2014). It is positioned on chromosome 10 in rats while chromosome 11 and chromosome 17 are for mice and human beings, respectively (Maharshak et al., 2013). MiR-132 has tissue specificity and has high expressions in nerve-

Table II				
Serum microRNA-132 expression in higher HCC patients				
Characteristics	n	Low (%)	High (%)	P value
Age (years)				
≥ 60	17	10 (58.8)	7 (41.2)	0.971
< 60	7	4 (57.1)	3 (42.9)	
Sex				
Female	9	6 (66.7)	3 (33.3)	0.588
Male	15	9 (60.0)	6 (40.0)	
HBsAg				
Positive	20	11 (55.0)	9 (45.0)	0.361
Negative	4	3 (75.0)	1 (25.0)	
Liver cirrhosis				
Positive	19	9 (47.4)	10 (52.6)	0.021
Negative	5	5 (100)	0 (0)	
TNM staging				
I	7	6 (85.7)	1 (14.3)	0.001
II	6	5 (83.3)	1 (16.7)	
III-IV	11	3 (27.3)	8 (72.7)	
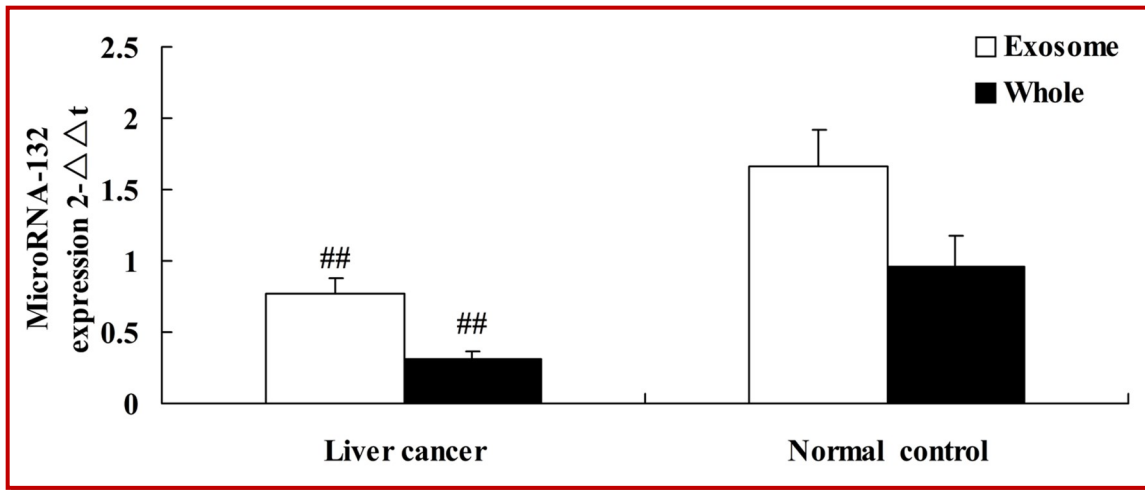
Figure 1: Correlations of microRNA-132 expression and patient clinicopathologic characteristics ##p<0.01 compared with normal group
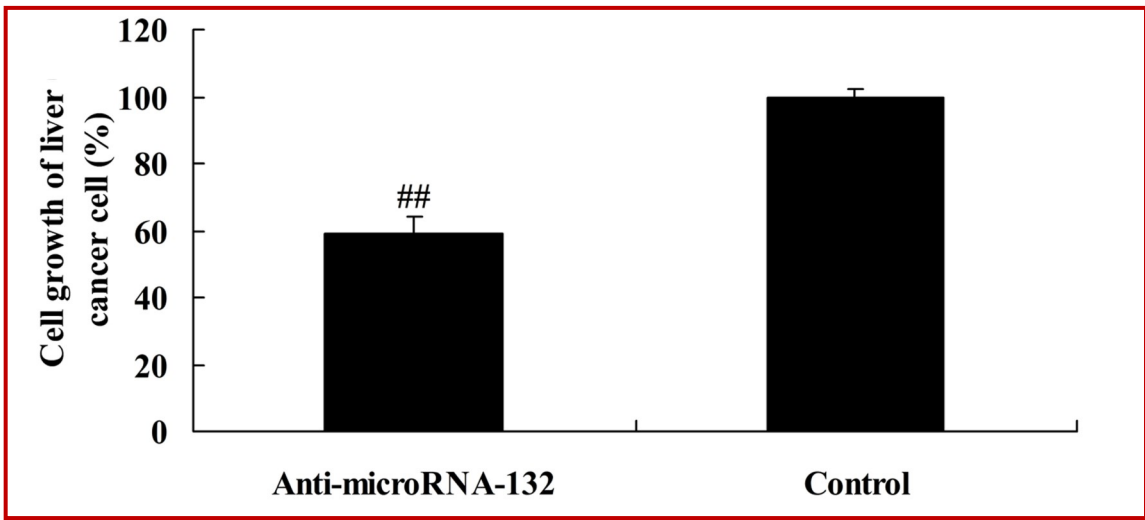

Figure 2: Mediation of microRNA-132 on cell growth of liver cancer cell; ##p<0.01 compared with normal group

related tissues (Wang et al., 2013). It participates in axon growth, proliferation and differentiation of synapsis and formation of nerve tumors. It also takes part in regulating cancers, neurodegenerative diseases, schizo-phrenia and immunological diseases (Wanet et al., 2012). In present study, there was significant suppre-ssion in microRNA-132 expression of exosome and whose liver cancer cell. Meanwhile, microRNA-132 expression of exosome cell was higher than that of whose cell in liver cancer cell or normal control group.

Expression of miR-132 is regulated by promoters and play significant part in metastasis and colony formation of tumor cells (Wei et al., 2013). Studies showed that upstream stimulatory factor-1 can increase expressions of miR-132 by incorporating with promoters of miR-132 (Kumarswamy et al., 2014). As a result, gene silencing of USF-1 can lead to down regulation of miR-132, then to avoid apoptosis of HepG2 cells under the conditions of oxygen-glucosede privation, which can be regarded

as the therapeutic target (Wang et al., 2014). In carcinoma of prostate cells, methylation of CpG island could down regulate miR-132 which controls cell adhesion by directly acting on protein-heparin-binding epidermal growth factor and talin 2, thus prevent the metastasis of carcinoma of prostate (Wei et al., 2013). We found that the down-regulation of microRNA-132 could inhibit cell growth and activated apoptotic of liver cancer cell.

Conclusion

We observed cases liver cirrhosis of low microRNA-132 expression was higher than that of high microRNA-132 expression. There was significant suppression in microRNA-132 expression of exosome and whose liver cancer cell. Down-regulation of microRNA-132 could inhibit cell growth and activated apoptotic of liver cancer cell. Furthermore, our study indicated that
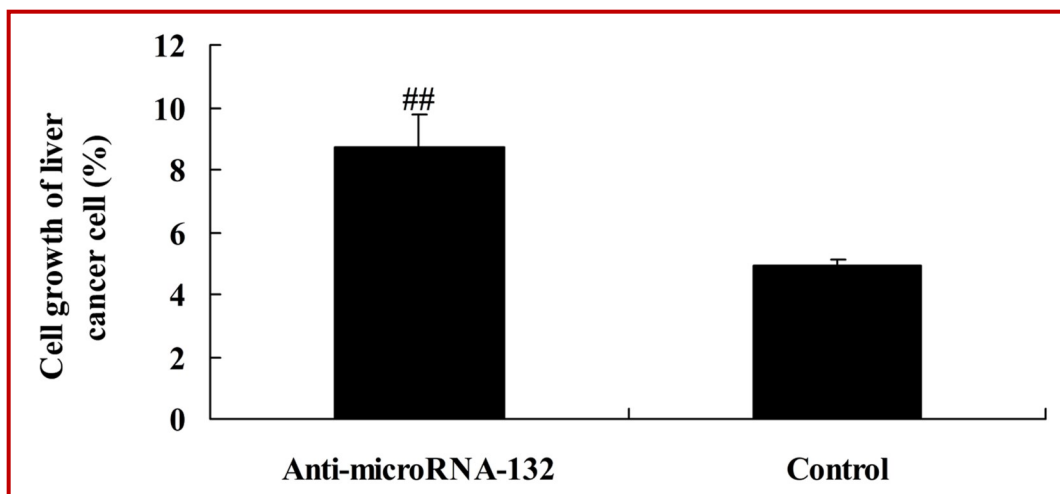
Figure 3: Mediation of microRNA-132 on apoptotic of liver cancer cell; ##p<0.01 compared with normal group

microRNA-132 provides increased sensitivity of detection than normal patients. MicroRNA-132 may serve as a potential biomarker for treatment on liver cancer.

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Ethical Issue

The study was approved by the Institutional Review Board of Hospital Ethics Committee. Written consents were obtained from all subjects prior to the recruitment.

Conflict of Interest

Authors declare no conflict of interest

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