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ORIGINAL ARTICLE

Expression changes of miRNA-203 before and after transcatheter arterial chemoembolization in hepatocellular carcinoma patients and its clinical significance

Xianglu Li[1]*, Shizong Li[2]*, Xuehua Xing[1], Lin Wang[1]

[1]Ward 1 of Oncology Department, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou, China. [2]Department of Hepatobiliary and Pancreatic Surgery, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou, China.

*Xianglu Li and Shizong Li contributed equally to this work

Summary

Purpose: *We aimed to detect expression changes of miRNA-203 in the serum of hepatocellular carcinoma (HCC) patients before and after transarterial chemoembolization (TACE), and to explore the clinical significance in influencing the prognosis of HCC.*

Methods: *Serum levels of miRNA-203 in HCC patients (n=100) and healthy subjects (n=100) were detected by RT-PCR. The relationship between miRNA-203 level and baseline characteristics of HCC was analyzed by Pearson correlation test. The prognostic potentials of miRNA-203 in HCC were evaluated by Kaplan-Meier method. Changes in miRNA-203 level before and after TACE in HCC patients were determined. Cox regression model was applied for analyzing potential factors that may influence the prognosis in HCC.*

Results: *MiRNA-203 was lowly expressed in the serum of HCC patients than in controls. Its level was closely linked to differentiation, metastasis and TNM stage. Serum level of miRNA-203 in HCC patients was upregulated following TACE. Overall survival was better in HCC patients expressing high level of miRNA-203 at post-TACE. Age (over 60 years), large tumor size (≥ 5 cm), metastasis and stage III-IV were risk factors of HCC death, while highly expressed miRNA-203 was a favorable factor.*

Conclusions: *Serum level of miRNA-203 is downregulated in HCC patients, which is upregulated following TACE. MiRNA-203 can be used to evaluate the prognosis in TACE-treated HCC patients.*

Key words: *MiRNA-203, HCC, TACE, prognosis, biomarker*

Introduction

Hepatocellular carcinoma (HCC) is a highly prevalent malignant tumor, and its mortality ranks third among malignant tumors [1]. Most HCC cases are already in the middle and advanced stages when diagnosed. Only about 20% HCC patients can be treated by surgery, radiofrequency ablation, and liver transplantation [2]. The therapeutic efficacy of surgical procedures in HCC treatment is not ideal, with postoperative 5-year recurrence rate of 65-75% [3]. A great number of HCC patients develop metastases and therefore lose the re-treatment opportunity [3]. It is necessary to find biomarkers that can reflect tumorigenesis and development of HCC [4].

Transcatheter arterial chemoembolization (TACE) is one of the main palliative treatments

Corresponding author: Shizong Li, MM. Department of Hepatobiliary and Pancreatic Surgery, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, No. 19, Xiuhua Rd, Haikou, Hainan, 570311, China.
Tel: +86 015607663959, Email: lishizongshanda@163.com
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for HCC. Iodized oil applied during the process of TACE can embolize blood supply vessels of HCC for a long period, thus achieving the anti-cancer effect of TACE. At the same time, chemotherapy drugs used in TACE can slowly and directly affect HCC foci [5]. So far, effective assessment of postoperative efficacy of TACE and monitoring relapse or metastasis of HCC are well concerned [6,7].

MicroRNAs (miRNAs) are 22 nucleotide long non-coding RNAs with endogenous regulatory functions, which are mainly distributed in eukaryotes [8,9]. In addition, abnormally expressed miRNAs are vital regulators in pathological progresses [9-11]. A relevant report demonstrated that miRNAs largely influence cell phenotypes and angiogenesis in HCC [12]. El-Halawany et al [13] identified a series of miRNAs that are differentially expressed in HCC patients before and after TACE. These miRNAs play an important role on assessing the therapeutic efficacy of TACE in HCC patients [14].

MiRNA-203 is an epithelial-specific, cancer-associated miRNA, which is located on human chromosome 14q32-33. It assists skin cells to build a protective layer on human squamous epithelial tissues, such as cervix, esophagus, bladder, etc. Besides, miRNA-203 extensively participates in regulating tumor cell behaviors as an oncogene or a tumor suppressor [15,16]. Yin et al [17] pointed out that miRNA-203 level is clearly connected to gastric cancer development. The potential role of miRNA-203 in influencing TACE efficacy in HCC patients remains largely unclear. This study aimed to detect the serum level changes of miRNA-203 in HCC patients before and after TACE, and to clarify its clinical significance.

Methods

Subjects

Clinical data of HCC patients treated at Hainan General Hospital from June 2014 to May 2016 were retrospectively analyzed. They were pathologically diagnosed as HCC by imaging examination or tissue biopsy. After standard admission examinations and tests, HCC patients had TACE performed. All recruited subjects signed written informed consent about this trial and surgical procedures. This study was approved by the ethics committee of Hainan General Hospital.

Inclusion criteria: (1) Definite clinical diagnosis; (2) No contraindications.

Exclusion criteria: (1) Patients had other treatment except for TACE, such as chemotherapy, liver resection, liver transplantation or ablation surgery; (2) Patients had TACE for other reasons except for HCC; (3) Patients had tumor metastases.

TACE protocols

TACE was conducted with catheterization of the femoral artery with Seldinger technique. Intubation in proper hepatic artery was performed by the guidance of X-ray and iohexol was administrated for angiography of tumor vessels. According to the distribution of tumor lesions, TACE was performed in the selected branches of blood supply arteries. Infusion chemotherapy drugs were 15 mg hydroxycamptothecin (HCPT) combined 30 mg epirubicin (EPI) or 30 mg pirarubicin (THP-ADM). For embolic agent, 10-30 ml of superliquidized iodized oil (38%) mixed with 1-2 ml of microspheres (100-700 μm in diameter) was applied, which was adjusted according to the tumor number, tumor size and embolic vessel diameter. There was at least an interval of 1-2 months between two performances of TACE. Antiemetics, acid suppression, liver protection and other symptomatic treatments were conducted after TACE.

Blood sample collection

6 mL of venous blood was taken in the morning from each subject under fasting state. The blood of HCC patients was taken for blood tests 1-3 days before TACE and one month after TACE, respectively. Blood samples were centrifuged at 4°C, 3,600 r/min for 15 min, and the upper layer serum was purified and stored at -80°C.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

TRIzol method (Invitrogen, Carlsbad, CA, USA) was applied for isolating RNAs from serum samples. Through reverse transcription of RNA at 16°C for 30 min, 42°C for 30 min and 85°C for 5 min, the extracted complementary DNA (cDNA) was used for PCR detection by SYBR Green method (TaKaRa, Tokyo, Japan). PCR reactions were 3-min pre-denaturation at 94°C, followed by 40 cycles at 94°C for 20 s, 60°C for 20 s and 56°C for 40 s. Each experiment was performed in triplicate. Primer sequences were listed as follows. MiRNA-203, F: 5'-TGGGGTGGGTGTGTCCAGC-3', R: 5'-GCTTCGGCAGCACATATACTAAAAT-3'; U6, F: 5'-AGGTCGGTGTGAACGGATTTG-3', R: 5'-GGGGTCGTTGATGGCAACA-3'.

Follow-up

HCC patients were followed up through telephone, outpatient review, hospitalized investigation or other methods for 5 years with 3-6 months interval. The therapeutic efficacy of HCC was assessed by performing enhanced CT in our hospital. Follow-up was terminated until death or the deadline.

Statistics

SPSS 20.0 software (IBM, Armonk, NY, USA) was used for all statistical analyses. Data were expressed as mean ± standard deviation (SD). The t-test was performed for analyzing differences between groups. Pearson correlation test was conducted to explore the relationship between miRNA-203 level and baseline characteristics of HCC patients. Prognostic potentials were assessed by Kaplan-Meier method. Cox regression model was applied to analyze risk factors of HCC. $P<0.05$ indicated significant difference.

Results

Baseline characteristics

In recruited 100 HCC patients, there were 48 males and 52 females, with average age 61.33 ± 15.06 years. A total of 53 males and 47 females were recruited in the control group, with average age of 59.82 ± 13.58 years. Baseline characteristics, including sex ratio, age and other indicators were comparable between groups (p>0.05) (Table 1).

Differential serum level of miRNA-203 in HCC patients

Serum level of miRNA-203 in each subject was determined by RT-PCR. Lower serum level of miRNA-203 was found in HCC patients than those of healthy controls (Figure 1A). A remarkable elevation of miRNA-203 level was seen after TACE (p<0.05) (Figure 1B), suggesting that miRNA-203 could reflect the therapeutic efficacy of TACE in HCC patients.

The relationship between miRNA-203 level and baseline characteristics of HCC

Pearson correlation test was conducted to explore the relationship between miRNA-203 level and baseline characteristics of HCC patients. Lower level of miRNA-203 was observed in HCC patients with large tumor size (≥ 5 cm), moderate or high level of differentiation, metastasis or stage III-IV (p<0.05). However, its level was unrelated to age and sex in HCC patients (p>0.05) (Table 2). It was concluded that miRNA-203 level may influence tumor size, differentiation, metastasis status and TNM staging in HCC.

Prognostic potential of serum miRNA-203 in HCC

Postoperative follow-up lasted 5-60 months. The median survival of recruited HCC patients was 33.6 months (mean 27.1). Based on the median serum level of miRNA-203 in HCC patients before TACE, they were classified to high miRNA-203 group and low miRNA-203 group. Kaplan-Meier survival curves revealed that the overall survival was higher in high miRNA-203 group than the other group (p=0.0253) (Figure 2A). In a similar way, HCC patients were classified to high miRNA-203 group and low miRNA-203 group based on the postoperative median serum level of miRNA-203. Consistently, patients in high miRNA-203 group had a better overall survival than the other group (HR=7.295, p=0.0069) (Figure 2B). It was concluded that the high level of miRNA-203 improved by TACE was conductive to the prognosis of HCC patients.

Table 1. Comparison of baseline characteristics of two groups

Variables	*Control (n=100)*	*HCC (n=100)*	x^2/t	*p*
Age, years	59.82 ± 13.58	61.33 ± 15.06	-0.745	0.457
Sex				
Male	48	53	0.5	0.572
Female	52	47		

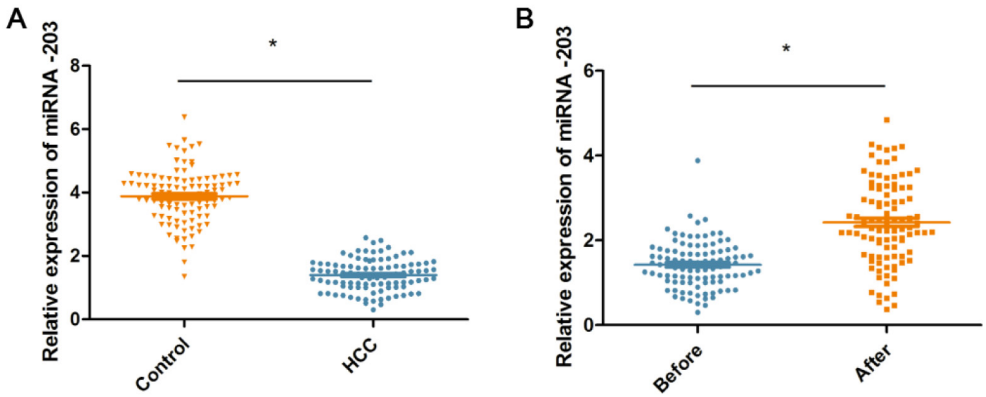

Figure 1. miRNA-203 in HCC patients and healthy subjects and expression changes of miRNA-203 before and after TACE. **A:** qRT-PCR data showed lower serum level of miRNA-203 in HCC patients than in controls. **B:** qRT-PCR data showed increased serum level of miRNA-203 in HCC patients at post-TACE (*p<0.05).

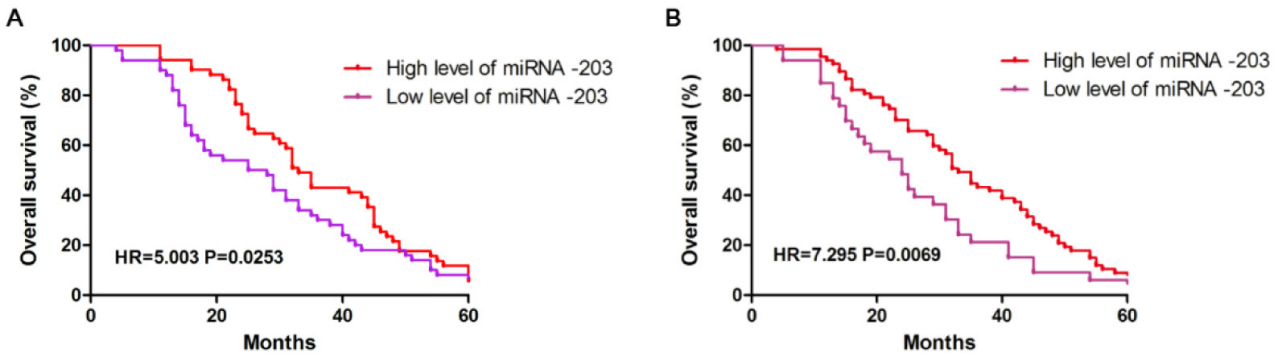

Figure 2. Diagnostic potential and prognostic potential of serum miRNA-203 in HCC. **A:** Kaplan-Meier survival curves revealed that the overall survival was higher in high miRNA-203 group than in low miRNA-203 (p=0.0253). **B:** The included 100 HCC patients were divided into two groups based on the median level of miRNA-203 detected one month after TACE. Overall survival was better in HCC patients expressing high level of miRNA-203 at post-TACE (HR=7.295, p=0.0069).

Table 2. The relationship between miRNA-203 level and baseline characteristics of HCC

Variables	*n*	*MiRNA -203 level*	*t*	*p*
Age (years)				
<60	55	2.67±1.15	0.045	0.964
≥60	45	2.66±1.03		
Sex				
Male	63	3.04±1.59	1.761	0.081
Female	37	2.51±1.18		
Tumor size (cm)				
<5	62	2.98±1.41	3.485	0.001
≥5	38	2.08±0.94		
Differentiation				
Moderate/High	59	1.86±0.83	4.65	<0.001
Low	41	3.13±1.85		
Metastasis				
Yes	44	2.01±1.14	2.169	0.033
No	56	2.64±1.64		
TNM stage				
I-II	52	2.87±1.53	2.66	0.009
III-IV	48	2.13±1.22		

Risk factors of HCC

Cox regression analysis uncovered that aged over 60 years, large tumor size (≥ 5 cm), metastasis and stage III-IV were risk factors of HCC death, with the HR of 2.624, 2.118, 3.004, 1.502, 2.117 and 1.937, respectively. Highly expressed miRNA-203 was a protective factor of HCC (HR=0.782, p<0.05) (Table 3).

Discussion

MiRNAs have been gradually applied in tumor treatment [18]. MiRNAs are differentially expressed between tumor tissues and normal ones [19,20] and their abnormal profiling can directly influence tumor development [21]. Owing to their specific expression patterns, miRNAs may be promising tumor hallmarks [8]. Most studies detected abnormally expressed miRNAs in histological samples of HCC, with limited clinical significance of miRNAs as tumor hallmarks. MiRNAs are highly stable in the human body fluids (i.e. blood, urine, etc.) and they are highly specific in disease progression [4]. They can be non-invasive indicators of tumors. In addition, expression changes of certain miRNAs before and after treatment provide a new direction in developing cancer recurrence and prognosis predictors.

Table 3. Risk factors of HCC analyzed by Cox regression model

Variables	*HR*	*95%CI*	*p*
Age (years)			
<60	1		
≥60	2.624	1.228-4.681	0.028
Sex			
Male	1		
Female	1.824	0.862-2.508	0.224
Tumor size (cm)			
<5	1		
≥5	1.502	1.149-3.377	0.008
Differentiation			
Moderate/High	1		
Low	3.078	0.537-4.068	0.373
Metastasis			
No	1		
Yes	2.117	1.436-3.084	0.003
TNM stage			
I-II	1		
III-IV	1.937	1.345-4.542	0.001
MiRNA-203 level			
Low	1		
High	0.782	0.221-0.924	<0.001

The abnormally expressed miRNA-203 possibly results from the abnormal regulation of CpG island methylation in its promoter region [22]. Abnormal hypermethylation of CpG island may cause transcriptional inactivation of tumor suppressor genes, thereby silencing their expressions. Conversely, abnormal hypomethylation of CpG island may activate proto-oncogenes, thereby inducing cell cancerization. Downstream genes of miRNA-203 are abundant [23]. MiRNA-203 is multi-targeted for downstream regulations. The complicated network formed by miRNA-203 and multiple downstream genes is responsible for cell phenotype regulations. It is reported that miRNA-203 is downregulated in colorectal cancer tissues and cell lines [24]. In this paper, miRNA-203 was lowly expressed in serum of HCC patients, indicating a potential anti-cancer role. Furthermore, miRNA-203 level was found to be related to differentiation, metastasis and TNM staging in HCC. Kaplan-Meier curves proved the prognostic potentials of miRNA-203 in HCC.

HCC patients in very early or early stage can be treated by surgical resection, local radiofrequency ablation, or liver transplantation. However, for those in middle or advanced stage, TACE is preferred [13]. MiRNA-203 expressions were detected in HCC patients 1-3 days before TACE administration and one month after, respectively. Interestingly, miRNA-203 was markedly upregulated following TACE. Meanwhile, high level of miRNA-203 at post-TACE was favorable to the prognosis in HCC patients. Cox regression analysis also confirmed that highly expressed miRNA-203 was a protective factor of HCC. To sum up, miRNA-203 was a prognostic hallmark of HCC. It could also be applied to monitor the therapeutic efficacy of TACE in HCC patients.

Conclusions

Serum level of miRNA-203 is downregulated in HCC patients, which is upregulated following TACE. MiRNA-203 can be used to evaluate the prognosis in TACE-treated HCC patients.

Conflict of interests

The authors declare no conflict of interests.

References

1. Rawla P, Sunkara T, Muralidharan P, Raj JP. Update in global trends and aetiology of hepatocellular carcinoma. Contemp Oncol (Pozn) 2018;22:141-50.
2. Xu R, Wang J, Huang X et al. Clinical value of spectral CT imaging combined with AFP in identifying liver cancer and hepatic focal nodular hyperplasia. JBUON 2019;24:1429-34.
3. Cheung TT, Han HS, She WH et al. The Asia Pacific Consensus Statement on Laparoscopic Liver Resection for Hepatocellular Carcinoma: A Report from the 7th Asia-Pacific Primary Liver Cancer Expert Meeting Held in Hong Kong. Liver Cancer 2018;7:28-39.
4. Ye JZ, Wang YY, Bai T et al. Surgical resection for hepatocellular carcinoma with portal vein tumor thrombus in the Asia-Pacific region beyond the Barcelona Clinic Liver Cancer treatment algorithms: a review and update. Oncotarget 2017;8:93258-78.
5. Gross A, Albrecht T. Transarterial Chemoembolisation (TACE) with Degradable Starch Microspheres (DSM) and Anthracycline in Patients with Locally Extensive Hepatocellular Carcinoma (HCC): Safety and Efficacy. Cardiovasc Intervent Radiol 2020;43:403-10.
6. Wang X, Zhang A, Sun H. Power of metabolomics in diagnosis and biomarker discovery of hepatocellular carcinoma. Hepatology 2013;57:2072-7.
7. Marquardt JU, Galle PR, Teufel A. Molecular diagno-

sis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. J Hepatol 2012;56:267-75.

8. Vishwakarma S, Agarwal R, Goel SK et al. Altered Expression of Sphingosine-1-Phosphate Metabolizing Enzymes in Oral Cancer Correlate With Clinicopathological Attributes. Cancer Invest 2017;35:139-41.
9. Hsu KW, Fang WL, Huang KH et al. Notch1 pathway-mediated microRNA-151-5p promotes gastric cancer progression. Oncotarget 2016;7:38036-51.
10. Mao B, Xiao H, Zhang Z, Wang D, Wang G. MicroRNA21 regulates the expression of BTG2 in HepG2 liver cancer cells. Mol Med Rep 2015;12:4917-24.
11. Lou Z, Gong YQ, Zhou X, Hu GH. Low expression of miR-199 in hepatocellular carcinoma contributes to tumor cell hyper-proliferation by negatively suppressing XBP1. Oncol Lett 2018;16:6531-9.
12. Ghosh A, Dasgupta D, Ghosh A et al. MiRNA199a-3p suppresses tumor growth, migration, invasion and angiogenesis in hepatocellular carcinoma by targeting VEGFA, VEGFR1, VEGFR2, HGF and MMP2. Cell Death Dis 2017;8:e2706.
13. El-Halawany MS, Ismail HM, Zeeneldin AA et al. Investigating the pretreatment miRNA expression patterns of advanced hepatocellular carcinoma patients in association with response to TACE treatment. Biomed Res Int 2015;2015:649750.
14. Yin XZ, Zhao DM, Zhang GX, Liu L. Effect of miRNA-203 on cervical cancer cells and its underlying mechanism. Genet Mol Res 2016;15:gmr8680.
15. Zhao G, Guo Y, Chen Z et al. miR-203 Functions as a Tumor Suppressor by Inhibiting Epithelial to Mesenchymal Transition in Ovarian Cancer. J Cancer Sci Ther 2015;7:34-43.
16. Melar-New M, Laimins LA. Human papillomaviruses modulate expression of microRNA 203 upon epithelial differentiation to control levels of p63 proteins. J Virol 2010;84:5212-21.
17. Yin LK, Sun XQ, Mou DZ. Value of Combined Detection of Serum CEA, CA72-4, CA19-9 and TSGF in the Diagnosis of Gastric Cancer. Asian Pac J Cancer Prev 2015;16:3867-70.
18. Wang P, Deng Y, Fu X. MiR-509-5p suppresses the proliferation, migration, and invasion of non-small cell lung cancer by targeting YWHAG. Biochem Biophys Res Commun 2017;482:935-41.
19. Chen M, Liu Y, Varley P et al. High-Mobility Group Box 1 Promotes Hepatocellular Carcinoma Progression through miR-21-Mediated Matrix Metalloproteinase Activity. Cancer Res 2015;75:1645-56.
20. Yang CH, Pfeffer SR, Sims M et al. The oncogenic microRNA-21 inhibits the tumor suppressive activity of FBXO11 to promote tumorigenesis. J Biol Chem 2015;290:6037-46.
21. Tan YS, Kim M, Kingsbury TJ, Civin CI, Cheng WC. Regulation of RAB5C is important for the growth inhibitory effects of MiR-509 in human precursor-B acute lymphoblastic leukemia. PloS One 2014;9:e111777.
22. Lohcharoenkal W, Das MK, Pasquali L et al. Genome-Wide Screen for MicroRNAs Reveals a Role for miR-203 in Melanoma Metastasis. J Invest Dermatol 2018;138:882-92.
23. Xu Z, Zhou Y, Shi F et al. Investigation of differentially-expressed microRNAs and genes in cervical cancer using an integrated bioinformatics analysis. Oncol Lett 2017;13:2784-90.
24. Li Z, Du L, Dong Z et al. MiR-203 suppresses ZNF217 upregulation in colorectal cancer and its oncogenicity. PLoS One 2015;10:e116170.