

Original Article

Prognostic significance of microRNA-203 in cholangiocarcinoma

Jingjing Li^{1*}, Beibei Gao^{2*}, Zufa Huang³, Tong Duan⁴, Daiqiang Li², Sheng Zhang³, Yujun Zhao³, Lian Liu³, Qiang Wang³, Zhizhao Chen³, Ke Cheng³

¹Department of Pathology, Xiangya Basic Medical College, Central South University, Changsha 410013, China;

²Department of Pathology, ⁴General Surgery, The Second Xiangya Hospital of Central South University, Changsha 410013, China; ³Transplantation Center, The Third Xiangya Hospital of Central South University, Changsha 410013, China. *Equal contributors and co-first authors.

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Abstract: MicroRNA functions as a tumor suppressor or a promoter in cholangiocarcinoma (CCA). Researchers have found that miR-203 functioned as tumor suppressor in many types of cancer. However, the role of miR-203 that plays in CCA remains to be clarified. We aimed to detect the expression level and the prognostic significance of miR-203 in CCA tissues. qRT-PCR was performed to examine the miR-203 expression levels in CCA tissue specimens and corresponding normal tissues. Our findings suggest that miR-203 expression was an independent poor prognostic factor for CCA patient overall survival. Therefore, miR-203 may serve as a valuable prognostic marker and promising treatment target for CCA.

Keywords: microRNA, miR-203, cholangiocarcinoma, prognosis

Introduction

cholangiocarcinoma (CCA) is a highly malignant cancer of the biliary tract with a poor prognosis [1-4]. According to the statistics, the incidence and mortality of CCA is rising worldwide [5]. CCA is difficult to diagnose in early-stage and characterized by high frequency of recurrence and metastasis. What's more, there is no effective chemoprevention or treatment, resulting in poor survival [6].

MicroRNAs (miRNAs) are non-coding RNAs of 20-22 nucleotides and regulate the translational inhibition of target mRNAs by base-pairing with their 3'-untranslated region (3'-UTR) [7]. Over the past few decades, a growing evidence suggests that an important role of miRNAs in various types of cancers [8]. In previous studies, researchers found that the expression of miR-203 was abnormal down-regulated has been observed in different types of cancers [9-11]. However, correlation between the expression of miR-203 and clinicopathological parameters in CCA patients have not yet been previously reported.

In this study, we firstly demonstrated that miR-203 expression was lower in CCA tissues compared with that in adjacent normal tissues. Next, we analyzed miR-203 expression level and its clinicopathological significance. Finally, in order to further investigate the predictor role of miR-203 in survival of CCA patients multivariate Cox regression analysis was used. Our findings will help to elucidate the roles of miRNAs in CCA.

Materials and methods

Patients

All patients had signed informed consent forms and the study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University. Clinical staging was performed according to the 6th edition 2002 American Joint Committee on Cancer (AJCC) TNM staging system. The selection criteria for patients with CCA were as follows: (1) the patients haven't received preoperative anti-tumor therapy. (2) Two pathologists confirmed

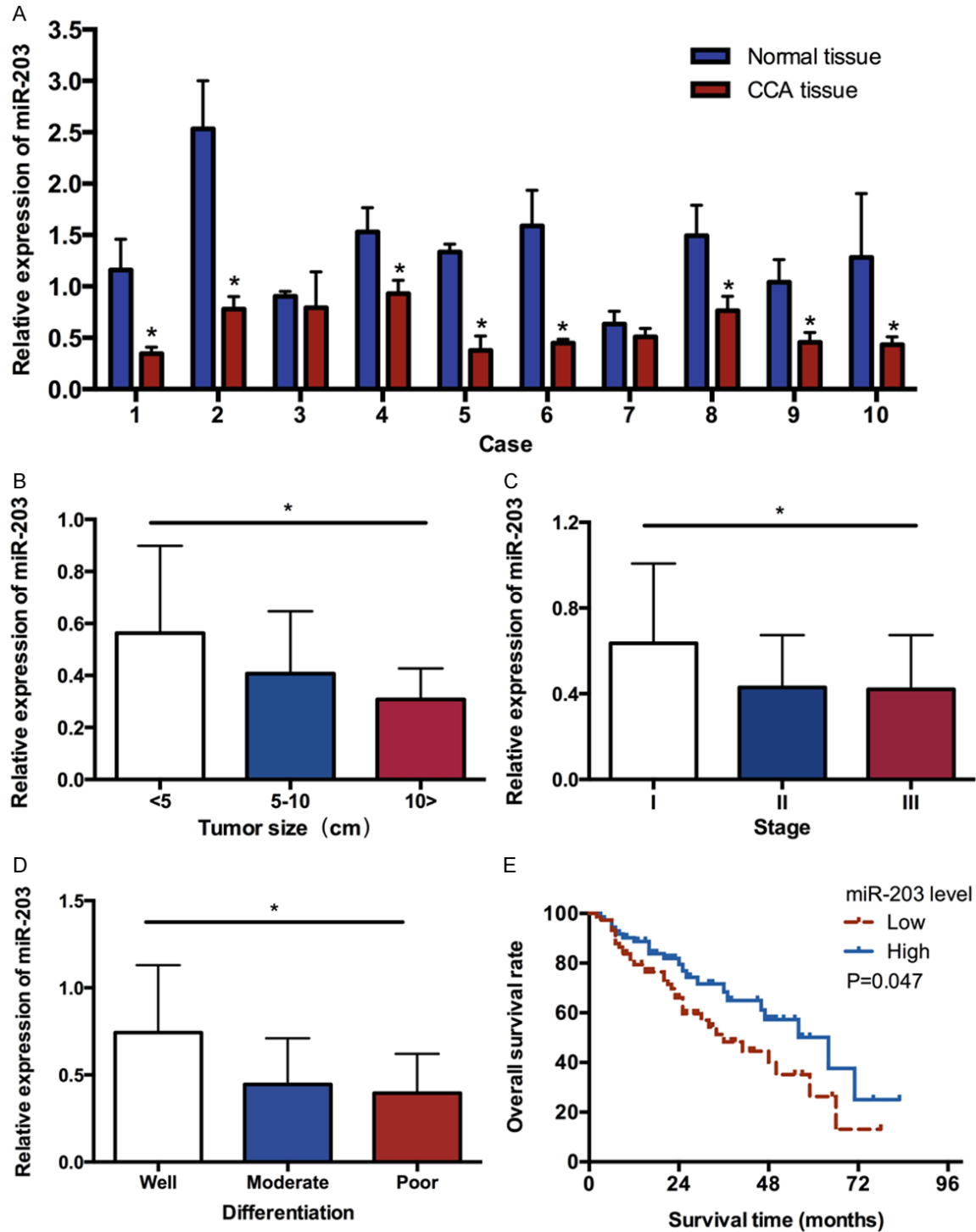


Figure 1. A. Comparison of miR-203 expression levels between CCA tissues and adjacent normal tissues. B. The expression level of miR-203 in CCA patients at different tumor size. C. The expression level of miR-203 in CCA patients at different clinical stages. D. The expression level of miR-203 at different differentiation of CCA tissues. E. Overall survival curve of CCA patients with different miR-203 expression. The patients with a lower expression of miR-203 had a lower survival time compared with those with a higher expression of miR-203. (* $P < 0.05$).

patients with CCA respectively. (3) The patients had no other cancer history. All patients were

diagnosed and treated between January 2006 and April 2014. All the clinical data of the

Table 1. Correlation between the expression of miR-203 and clinicopathological parameters in CCA patients

Characteristics	miR-203 expression		P value	
	High	Low		
Age	<55	41 (51.9%)	38 (48.1%)	0.621
	≥55	33 (47.8%)	36 (52.2%)	
Gender	Male	39 (49.4%)	40 (50.6%)	0.869
	Female	35 (50.7%)	34 (49.3%)	
Tumor size (cm)	<5	45 (60.8%)	29 (39.2%)	0.026*
	5~10	26 (40.6%)	38 (59.4%)	
	≥10	3 (30.0%)	7 (70.0%)	
Tumor number	Solitary	64 (52.9%)	57 (47.1%)	0.136
	Multiple	10 (37.0%)	17 (63.0%)	
Clining stage	I	26 (68.4%)	12 (31.6%)	0.030*
	II	20 (45.5%)	24 (54.5%)	
	III	28 (42.4%)	38 (57.6%)	
Differentiation	Well	18 (78.3%)	5 (21.7%)	0.003*
	Moderate	42 (50.0%)	42 (50.0%)	
	Poor	14 (34.1%)	27 (65.9%)	
Cholelithiasis	Yes	34 (57.6%)	25 (42.4%)	0.131
	No	40 (44.9%)	49 (55.1%)	
Cirrhosis	Yes	13 (39.4%)	20 (60.6%)	0.167
	No	61 (53.0%)	54 (47.0%)	
AFP	Normal	52 (48.1%)	56 (51.9%)	0.459
	Abnormal	22 (55.0%)	18 (45.0%)	

*: P<0.05.

patients were also collected respectively. The mean follow-up period was 26 months. Survival time was calculated from the date of the initial surgery to death.

Tissue specimens

Human CCA and corresponding normal tissues (located >2 cm away from the tumor) were obtained from 10 patients who underwent CCA resection at the Second Xiangya Hospital Central South University. The other 138 samples of CCA tissues were collected from the Pathology Department.

Quantitative real-time RT-PCR

miRNA from tissues and cells was extracted using TRIzol (Invitrogen) reagent according to the manufacture's instruction. Real-time qRT-PCR was carried out as described previously [12]. The qRT-PCR primers for miR-203 (HmiRQP4643) and U6 (HmiRQP1433) were purchased from Guangzhou Funeng Gene Co., Ltd (Guangzhou, China). The relative expression level of miR-203 was normalized to that of U6 by using the $2^{-\Delta\Delta Ct}$ cycle threshold method.

Statistical analysis

Statistical analyses were carried out using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Difference of measurement data was assessed by Student's t-test. Count data were analyzed by the χ^2 or Fishers exact tests. Univariate survival analysis was performed using Kaplan-Meier method and the Log-rank test. Multivariate survival analysis was performed using the Cox multivariate analysis model. $P<0.05$ was considered statistically significant.

Results

Expression levels of miR-203 in CCA

We used qRT-PCR to examine miR-203 expression in 10 pairs of CCA tissues and the corresponding noncancerous tissues. As shown in **Figure 1A**, the expression of miR-203 was significantly down-regulated in CCA tissues when compared with adjacent normal tissues. Since the miR-203 was in lower level compared with that in the matched normal tissues, we postulated that miR-203 may play an important role in CCA. To test this hypothesis we collected other 138 CCA tissue specimens to further analyze the relationship between miR-203 expression level and clinical characters of CCA patients.

Expression levels of miR-203 and clinicopathological characteristics in CCA

The miR-203 expression levels were classified as high or low in relation to the median value. Low expression of miR-203 was found to signifi-

Table 2. Multivariate analyses for overall survival by Cox regression model

	P value	Exp (B)	RR 95% CI	
			Lower	Upper
Age	0.48	1.23	0.692	2.188
Gender	0.094	1.645	0.918	2.945
Tumor size	0.311	1.264	0.803	1.988
Tumor number	0.201	0.581	0.253	1.334
Differentiation	0.020*	1.743	1.092	2.783
Clining stage	0.001*	2.188	1.383	3.462
Cirrhosis	0.077	1.835	0.935	3.602
Cholelithiasis	0.91	0.966	0.53	1.761
AFP	0.122	0.623	0.342	1.134
miR-203	0.017*	3.444	1.244	9.536

*: $P < 0.05$.

cantly correlate with tumor size (**Figure 1B**), clinical stage (**Figure 1C**) and tumor differentiation (**Figure 1D**), and it suggested that miR-203 might be closely related with the development of cholangiocarcinoma. However, there were no significant difference in miR-203 expression was observed with age, sex distribution, tumor number, cirrhosis, serum AFP value and cholelithiasis (shown in **Table 1**).

Low-expression level of miR-203 predicts poor prognosis in CCA patients

Considering that the level of miR-203 expression was remarkably correlated with tumor size, clinical stage and tumor differentiation, we hypothesized that miR-203 might affect the prognosis of CCA patients. Kaplan-Meier analysis with the log-rank test indicated that low miR-203 expression had a significant impact on OS (1 year, 3 year, and 5 year survival rate were 79.42%, 48.29%, and 26.33% vs. 88.71%, 60.27%, and 37.62%, respectively; $P < 0.05$; **Figure 1E**). Univariate and multivariate analyses were utilized to evaluate whether the miR-203 expression level and various clinicopathological features were independent prognostic parameters. Multivariate analysis revealed that miR-203 expression, clinical stage, tumor differentiation and cirrhosis were independently associated with the overall survival (shown in **Table 2**).

Discussion

A large number of studies suggest that miRNAs play a key role in tumor development. Increasing

evidence suggests that the dysregulation of miRNAs participates in CCA progression where they act as either suppressors or promoters at the post-transcriptional regulation stage. For example, Zhang J [13] *et al.* demonstrated that expression of miR-26a was higher in CCA tissues. miR-26a promotes CCA cell growth by inhibition of GSK-3 β and subsequent activation of β -catenin. MicroRNA-144 suppresses CCA cell proliferation and invasion through targeting platelet activating factor acetylhydrolase isoform 1b (LIS1) [14]. Wang LJ [15] *et al.* showed that high expression levels of miR-21 were closely related to adverse clinical features, diminished survival, and poor prognosis in CCA patients.

miR-203 has been shown to down-regulate expression and inversely correlate with BMI1 expression in melanoma and inhibit invasive and proliferative abilities in part by targeting BMI1 [16]. miR-203 enhances chemosensitivity to 5-fluorouracil by targeting thymidylate synthase in colorectal cancer [17]. Wang C [18] *et al.* demonstrated that miR-203 played a critical role in regulating cell proliferation, apoptosis and migration by targeting of PKC α in lung cancer cells. All these findings emphasize a fundamental role of miR-203 in suppressing malignant tumor.

In this study, our results showed that miR-203 expression was significantly lower in CCA tissues compared with normal adjacent tissues. The relationship of the miR-203 with various clinical features of CCA patients was analyzed. The results showed that a low level of miR-203 expression was significantly correlated with tumor size, clinical stage and tumor differentiation, suggesting that miR-203 might be closely related with tumor development and prognosis of CCA. Furthermore, the overall survival rate of low miR-203 expression group was significantly shorter than that of high miR-203 expression group. Finally, in a multivariate Cox model, we found that miR-203 expression was an independent poor prognostic factor for overall survival rate, indicating that low miR-203 level was significantly associated with poor OS in CCA patients.

In conclusion, the expression of miR-203 was dramatically decreased in CCA tissues. Low expression of miR-203 was significantly associated with tumor progression and predicted

poor prognosis in the CCA patients. These findings indicate that miR-203 serves as a novel prognostic marker and potential treatment target in CCA.

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Disclosure of conflict of interest

None.

Address correspondence to: Zhizhao Chen and Ke Cheng, Transplantation Center of The Third Xiangya Hospital of Central South University, Changsha 410013, China. Tel: +86-15116331665; Fax: +86-731-8921910; E-mail: czzxiangya@aliyun.com (ZZC); csuchengke@aliyun.com (KC)

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