

MiR-106B Represents an Innovative Bio-marker in Cholangiocarcinoma Detection

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Abstract

Background

Cholangiocarcinoma (CCA) is one of the most aggressive malignancies. Late diagnosis may be responsible for the high mortality. MicroRNA-106b (*MiR-106b*) is accepted as an important regulator in various human malignancies. The current study was aimed to investigate the diagnostic value of miR-106b in CCA.

Methods

Serum levels of *miR-106b* in CCA patients and healthy control were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). Chi-square test was used to analyze the association of *miR-106b* with the clinicopathological features. To evaluate the diagnostic value of *miR-106b* in CCA, the ROC curve was constructed.

Results

The expression of *miR-106b* was significantly increased in CCA samples compared with the healthy controls ($P < 0.001$). The overexpression of *miR-106b* was remarkably correlated with the lymphatic node metastasis ($P = 0.038$), clinical stage ($P = 0.017$) and differentiation ($P = 0.009$). ROC curve suggested that *miR-106b* was an effective diagnostic biomarker in CCA with the AUC of 0.913. The optimal cutoff value was 2.525, with the sensitivity of 89.7% and the specificity of 79.3%.

Conclusions

MiR-106b functions as an oncogene in CCA, which may be a potential diagnostic biomarker for CCA.

Background

Cholangiocarcinoma (CCA) is the second common primary hepatic malignancy, with the increased incidence around the world over recent decades [1, 2]. Based on the tumor location, CCA can be classified into two types, including intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECCA) [3]. Surgical resection is the optimal strategy for this deadly disease [4]. However, most of patients are diagnosed at an advanced stage, missing the optimal time for operation and leading to poor survival [5, 6]. Early detection is difficult in CCA, due to the silent nature of the cancer at early stage and the lack of effective diagnostic tools [7, 8]. The conventional blood-based biomarkers, such as carcinoembryonic antigen, carbohydrate antigen (CA) 19 - 9, CA242, exhibit low sensitivity and specificity in CCA diagnosis [9]. Therefore, finding novel diagnostic biomarkers for CCA is crucial to survival for patients with this disease.

MicroRNAs (miRNAs) are a group of small, single stranded non-coding RNAs with approximately 22 nucleotides in length. MiRNAs play important roles in various cellular and biological processes by regulating gene expression at transcriptional level [10, 11]. The aberrant expression of miRNAs is accepted a biomarker for several diseases like cancers [12]. For example, a related study scheduled by Wang et al. suggested that serum levels of *miR-26a* were significantly different between CCA patients and healthy individuals, which might serve as a indicator for CCA formation [13]. The expression profile of miRNAs shows a significant association with tumor development and progression, suggesting their potential as biomarkers for cancer diagnosis and prognosis [14].

MicroRNA-106b-25 cluster is located on the chromosome 7. This cluster has been reported to act as oncogenes for several cancers, such as hepatocellular carcinoma, prostate cancer, and gastric cancer [15–17]. MicroRNA-106b (*miR-106b*), belonging to microRNA-106b-25 cluster, is also a cancer-related gene. The abnormal expression of *miR-106b* was observed in some human cancers, including hepatocellular carcinoma, colonic cancer, breast cancer [18–20]. However, the relationship between *miR-106b* expression and CCA has been rarely reported.

In the present study, we aimed to assess the diagnostic significance of *miR-106b* in the CCA patients. The serum level of *miR-106b* was detected in CCA patients, as well as its association with clinical characteristics. The present study might explore a novel predictor for CCA diagnosis.

Methods

Patients and specimens collection

The permission for this study was obtained from the ethics committee of the PLA Rocket Force Characteristic Medical Center. All the individuals collected in this study provided the written informed consent before sampling.

156 patients who were pathologically diagnosed with CCA in the PLA Rocket Force Characteristic Medical Center were collected in the study. None of the patients had received chemotherapy or radiotherapy before blood collection. In addition, 58 healthy volunteers who visited the hospital for routine checkup and have no history of carcinoma were enrolled in the study as healthy controls. The patients group and healthy controls were age and gender matched.

5 ml blood specimens were collected from the participants, and stored in EDTA tubes. Then the samples were centrifuged at 2000 g for 15 min to separate serum specimens. The serum specimens were stored at -20°C for until use.

The clinicopathological features of CCA patients were recorded, including age, gender, tumor size, lymphatic node metastasis, clinical stage, histological type and differentiation. All these data were listed in Table 1.

Table 1
Correlation of *miR-106b* expression with clinicopathological characteristics of CCA patients

Characteristics	No. N = 156	<i>MiR-106b</i> expression		<i>P</i> values
		Low (n = 67)	High (n = 89)	
Age (years)				0.131
< 60	69	25	44	
≥ 60	87	42	45	
Gender				0.628
Male	85	38	47	
Female	71	29	42	
Tumor size (cm)				0.221
< 2	82	39	43	
≥ 2	74	28	46	
LN metastasis				0.038
Yes	87	31	56	
No	69	36	33	
Clinical stage				0.017
I-II	76	40	36	
III-IV	80	27	53	
Histological type				0.791
Nonpapillary	95	40	55	
Papillary	61	27	34	
Differentiation				0.009
Well	79	42	37	
Moderate/Poor	77	25	52	
Note: LN, lymphatic node.				

Rna Isolation And Quantitative Real-time Polymerase Chain Reaction (qrt-pcr)

Total RNA were isolated from collected serum samples using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. The concentration of RNA was measured by NanoDrop ND-1000 (NanoDrop, Waltham, MA). The reverse transcription was carried out by the AMV reverse transcription system (Promega, USA).

In this study, quantitative real-time polymerase chain reaction (qRT-PCR) was used to evaluate the expression of *miR-106b* in serum samples. This reaction was performed with SYBR Green PCR master mix (Applied Biosystems, USA) in 7300 Real-Time PCR System (Applied Biosystems, USA). *U6* gene was used as the endogenous control. All the primer sequences were as follows: *miR-106b* forward: 5'-TAAAGTGCTGACAGTGCAGAT-3', reverse: 5'-ATCTGCACTGTCAGCACTTTA-3'; *U6*, forward: 5'-TGCGGGTGCTCGCTTCGGCAGC3', reverse: 5'-CCAGTGCAGGGTCCGAGGT3'. Relative expression of *miR-106b* was normalized to *U6* and calculated by $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analyses were performed by the SPSS 21.0 statistical software. Student's t test was applied to compare the different expression of *miR-106b* between CCA patients and healthy controls. Chi-square test was used to analyze the correlation of *miR-106b* expression with the clinicopathological features. To evaluate the diagnostic performance of *miR-106b* in CCA, we constructed the ROC curve in the present study. In all analyses, $P < 0.05$ was considered statistically significant.

Results

The expression level of miR-106b

The expression levels of *miR-106b* in 156 CCA patients and 58 healthy volunteers were measured by qRT-PCR. The results suggested that the expression of *miR-106b* was significantly increased in the CCA patients compared with the healthy controls, and this differentiation was statistically significant ($P = 0.001$) (Fig. 1).

Association of miR-106b with clinicopathological characteristics in CCA patients

The patients diagnosed with CCA were divided into high expression group ($n = 89$) and low expression group ($n = 67$), according to their median serum levels of *miR-106b*. In addition, we examined the relationship between *miR-106b* levels and the clinicopathological data of CCA patients using chi-square test. The results revealed that *miR-106b* expression was significantly correlated with the lymphatic node metastasis ($P = 0.038$), clinical stage ($P = 0.017$) and differentiation ($P = 0.009$). However, there was no significant association between *miR-106b* expression and age, gender, tumor size or histological type (all $P > 0.05$) (Table 1).

Estimation of the diagnostic value of miR-106b in patients with CCA

In order to evaluate the diagnostic accuracy of *miR-106b* in CCA, ROC curve was constructed in this study. ROC curve showed that the area under the curve (AUC) was 0.913, suggesting the high diagnostic accuracy of *miR-106b* in CCA. The cutoff value of *miR-106b* for CCA diagnosis was 2.525, with the sensitivity of 89.7% and the specificity of 79.3% (Fig. 2).

Discussion

CCA is a highly malignant cancer, which originates from ductular epithelium of biliary tree [21]. Evidences show that the incidence and mortality have been on the rise, especially in several Southeast Asian countries [22]. Due to the asymptomatic nature and inability to early diagnosis, CCA patients are usually diagnosed at the advanced stage, leading the high mortality and poor prognosis [7]. What is worse, CCA is insensitivity to chemotherapy, making the treatments more difficult [23]. Thus, novel targets for diagnosis and treatments are urgently needed.

MiRNAs are a class of small and noncoding RNAs, which have been reported to be involved in the progression of various human cancers [24]. The expression levels of miRNAs are significantly correlated with tumor formation and development, which can serve as biomarkers for cancer diagnosis and prognosis [14]. In the previous tumor investigation, a variety of miRNAs were reported to be involved in CCA tumorigenesis. Wang et al. demonstrated that *miR-26b* as an oncogene could promote aggressive tumor progression of CCA, leading to poor survival [13]. Liu et al. reported that overexpression of *miR-122* in vitro could inhibit CCA cell proliferation, invasion and promote apoptosis, which might be a potential target for CCA diagnosis and treatments [25]. Based on the related analysis results, we speculated that miRNAs played important roles in tumorigenesis of CCA, which might improve the accuracy of early detection.

MiR-106b, a member of the *miR-106b-25* cluster, is proved to serve as an oncogene in several human cancers, including hepatocellular carcinoma [18], renal cell carcinoma [26], bladder cancer [27]. However, in gastric cancer and melanoma, *miR-106b* could inhibit malignant tumor development [28, 29]. All the related studies might reveal that *miR-106b* played distinct roles in various malignancies. In the present study, we examined the expression of *miR-106b* in CCA patients and healthy controls. The analysis results demonstrated that the expression of *miR-106b* was upregulated in CCA patients compared with healthy controls. Moreover, the elevated *miR-106b* expression was significantly associated with positive lymphatic node metastasis, advanced clinical stage and poor differentiation. These experimental data might suggest that miR-106 was an oncogene in CCA. The conclusion was consistent with the previous studies. Amy *et al.* reported that the *miR-106b* expression in the CCA samples was significantly higher than that in the normal samples [30]. The study carried out by Li et al. demonstrated that *miR-106b* could regulate biological behaviors of renal cell carcinoma cell, thus promoting cancer progression [26]. However, the mechanisms for *miR-106b* regulating CCA were not investigated in the present study. Further researches were needed.

MiR-106b has been reported to be a biomarker for several cancers. Staby et al. suggested that upregulated *miR-106b* predicted early metastasis for renal cell carcinoma patients who were treated with nephrectomy [31]. The study scheduled by Li et al. indicated that *miR-106b* might serve as a promising prognostic biomarker for hepatocellular carcinoma patients. The elevated levels of *miR-106b* predicted poor prognosis for the patients [32]. Zheng et al. reported that plasma level of *miR-106b* showed high accuracy in breast cancer diagnosis [33]. All the related researches might reveal that *miR-106b* was a useful biomarker for cancers. In the present study, ROC curve was constructed to evaluate the diagnostic value of *miR-106b* in CCA. Analysis results demonstrated that *miR-106b* could distinguish the CCA patients from healthy individuals with high sensitivity and specificity.

Conclusions

In summary, the expression level of *miR-106b* is upregulated in the CCA patients, and associated with aggressive clinical characteristics. *MiR-106b* may be a potential diagnostic biomarker for CCA.

List Of Abbreviations

Cholangiocarcinoma (CCA)

quantitative real-time polymerase chain reaction (qRT-PCR)

intrahepatic cholangiocarcinoma (ICCA)

extrahepatic cholangiocarcinoma (ECCA)

carbohydrate antigen (CA)

MicroRNAs (miRNAs)

MicroRNA-106b (*miR-106b*)

area under the curve (AUC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materialsThe datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.**Competing interests**The authors declare that they have no competing interests.**Funding** Not applicable.**Authors' contributions** N.W. and Y.L. conceived and designed the experiments; Y.Z. and H.C. conceived and performed the experiments; X.W. and Z.L. prepared figures. Z.L., X.W. and N.W. wrote the main manuscript text. All authors reviewed the manuscript.**Acknowledgements** Not applicable.

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Figures

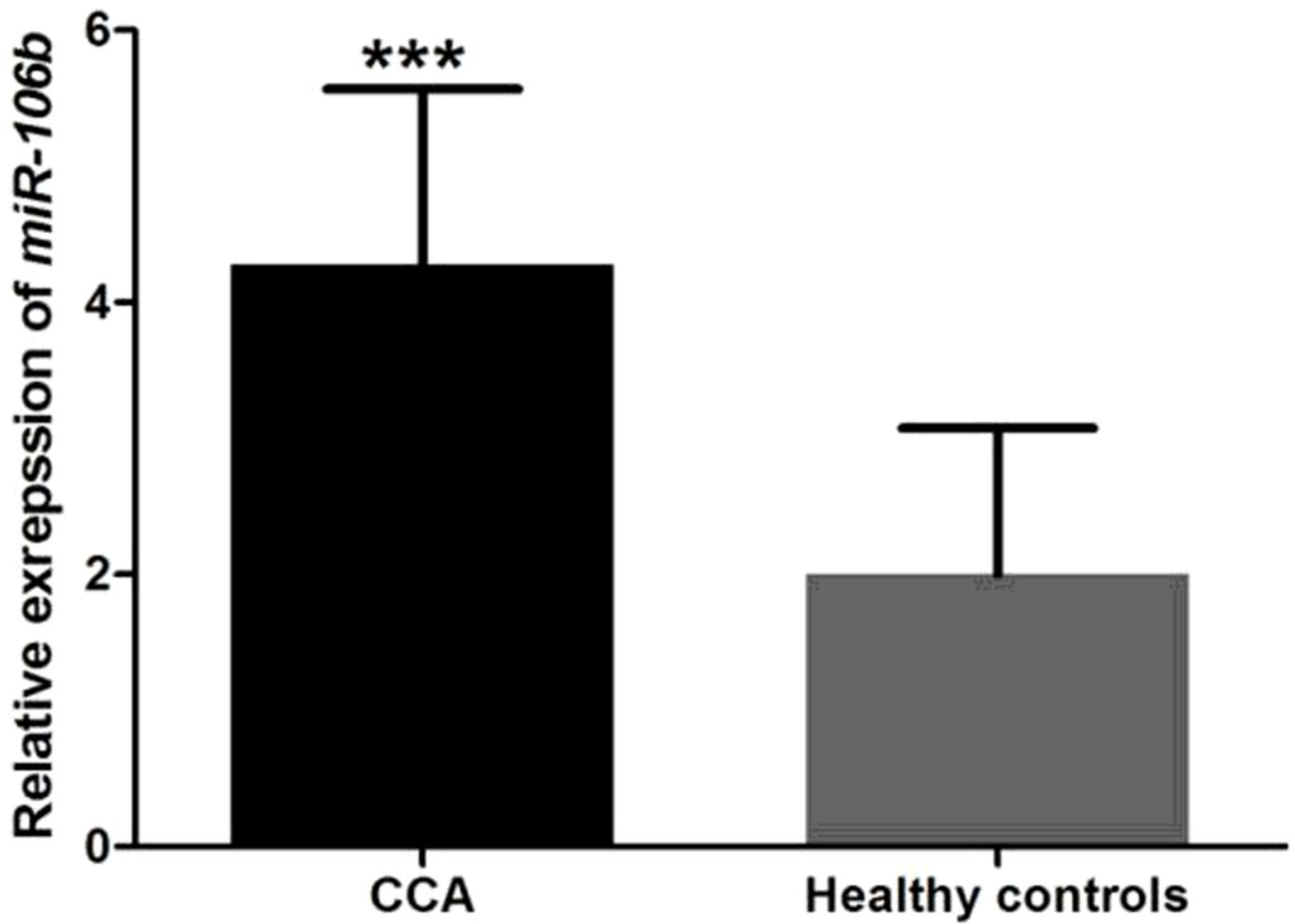


Figure 1

The relative expression level of miR-106b evaluated by qRT-PCR in CCA patients and healthy controls. Analysis results suggested that serum levels of miR-106b were significantly up-regulated in CCA patients, compared with healthy controls. ***: indicated $P < 0.001$.

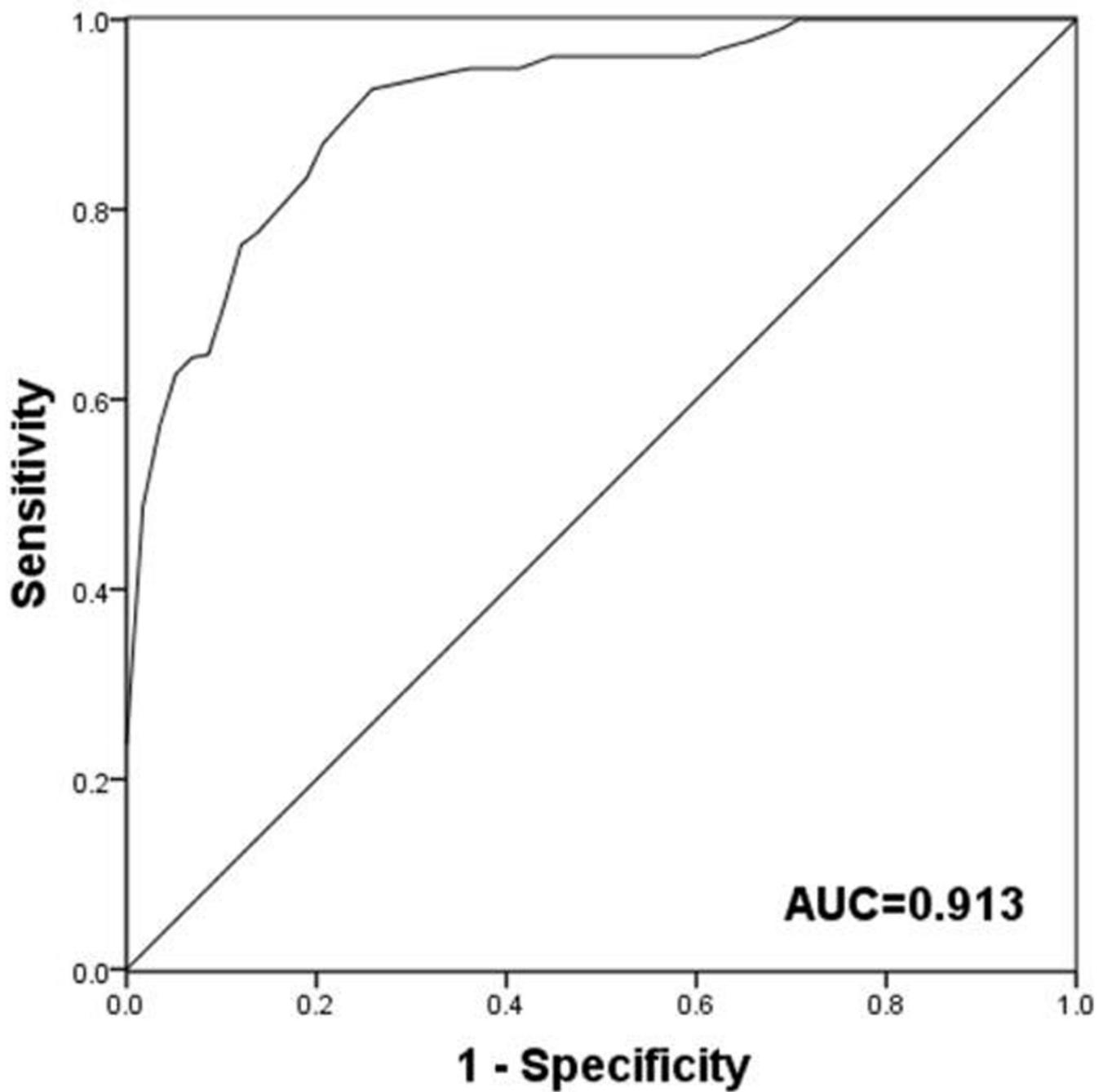


Figure 2

ROC analysis based on miR-106b expression for CCA patients. The curve demonstrated that miR-106b showed high diagnostic value for CCA patients, with the AUC value of 0.913, combining with the sensitivity of 89.7% and the specificity of 79.3%. The cut-off value of miR-106b for CCA diagnosis was 2.525.