MicroRNA-124 alone might represent an indicator signifying cholangiocarcinoma prognosis

Ning Wang

the PLA Rocket Force Characteristic Medical Center

Yanni Li

the PLA Rocket Force Characteristic Medical Center

Yanfang Zheng

the PLA Rocket Force Characteristic Medical Center

Huoming Chen

the PLA Rocket Force Characteristic Medical Center

Xiaolong Wen

University of Science and Technology Beijing

Zhaoxia Li (✉ shnfong@126.com)

the PLA Rocket Force Characteristic Medical Center <https://orcid.org/0000-0001-6993-5507>

Primary research

Keywords: MicroRNA-124, Cholangiocarcinoma, Survival, Prognosis

Posted Date: June 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-33111/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Previous studies have demonstrated that microRNAs (miRNAs) played a crucial role in various diseases, including cancers. The aim of the study was to evaluate the clinical significance of miR-124 in patients with cholangiocarcinoma (CCA).

Methods: The expression pattern of miR-124 was detected in CCA tissues using quantitative reverse transcription polymerase chain reaction (qRT-PCR). The correlation of miR-124 expression with clinicopathological features and overall survival of patients were explored using chi-square test, Kaplan-Meier methods and Cox regression analyses.

Results: The miR-124 expression level was strong down-regulated in CCA tissues compared with normal para-cancerous tissues ($P<0.001$). Moreover, aberrant miR-124 expression was significantly associated with differentiation ($P=0.045$) and lymph node metastasis ($P=0.040$). In addition, Kaplan-Meier method and log-rank test revealed that patients with low miR-124 expression has a poorer overall survival compared with those with high miR-124 expression ($P=0.002$). Furthermore, multivariate analysis confirmed that miR-124 expression ($P=0.006$; HR=2.006; 95%CI: 1.224-3.289) was an independent prognostic indicator in CCA.

Conclusions: Collectively, our results defined miR-124 expression plays important roles in CCA patients. MiR-124 expression might used as a valuable prognostic biomarker for patients with CCA.

Background

Cholangiocarcinoma (CCA) is a devastating malignancy derived from bile duct epithelial cells, and the mortality associated with CCA is currently increasing worldwide [1]. According to the statistics, the incidence of CCA changes widely among different geographic regions; the highest number of cases occurs in southeast Asia while the lowest number occurs in Australia [2]. Anatomically, CCA is categorized as intrahepatic cholangiocarcinomas (IHCCs) and extrahepatic cholangiocarcinoma (EHCCs), the latter being further divided into perihilar CCAs and distal CCAs [3]. CCA is difficult to diagnosis in early-stage due to its lack of early symptoms [4], and it is featured by high frequency of recurrence and metastasis. What's more, there is no effective chemoprevention or treatment, leading to an unfavorable prognosis [5].

MicroRNAs (miRNAs) are a type of endogenous coding small molecular RNAs with 18–25 nucleotides in length that could negatively control the translational inhibition of target mRNAs through base-pairing with their 3'-untranslated region (3'-UTR) [6, 7]. MicroRNAs are known to play an important role in diverse biological processes, such as apoptosis, cell proliferation, and differentiation [8], which displays their functionality in carcinogenesis as tumor suppressor genes or oncogenes [9–11].

miR-124 is one of the cancer-related miRNAs. It is a brain-enriched miRNA and was first discovered to be involved in stem cell regulation and neuro-development [12, 13]. Previous studies indicated that miR-124

is down-regulated in various cancers. For example, in colorectal cancer, miR-124 is strongly decreased, and promotes apoptosis of colorectal cells [14]. In addition, Li *et al.* revealed that miR-124 is reduced and negatively related to lymph node metastasis in breast cancer [15]. The above studies indicated miR-124 play a crucial role in tumorigenesis and progression. However, the clinical significance of miR-124 remains unknown in CCA.

In the present study, we measured the relative expression level of miR-124 in CCA tissues via qRT-PCR, and analyzed the relationship of miR-124 level with clinicopathological features. We also explored the prognostic performance of miR-124 in CCA.

Methods

Patients and tissues samples

The use of tissues for this study has been approved by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center. At the time of initial diagnosis, all patients had provided consent in the sense that their tumor samples could be used for investigation purposes. Written informed consents were received from all participants involved in the study.

In total, 119 frozen cancerous samples from CCA patients undergoing surgery from at our department were collected in this study. In addition, 119 matched para-cancerous samples were collected. All the specimens were diagnosed by two pathologists separately. None of the patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. The clinical features of the patients, including age, gender, tumor size, location, distant metastasis, differentiation, TNM stage, and lymph node metastasis were listed in **Table 1**. The overall survival time was computed as the time from the date of first pathologic diagnosis to the date of death or last follow-up.

RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA from fresh tissues was isolated using the mirVana miRNA Isolation kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. Single-stranded cDNA for microRNA analysis was also synthesized by RT using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. qRT-PCR method was used to assess the expression levels of miR-124 with TaqMan miRNA assay kit (Applied Biosystems, Foster, CA, USA) on a 7500 Fast Real-time PCR System (Applied Biosystems, California, USA). U6 RNA was used as an endogenous reference for normalizing the expression levels of miR-124. The threshold cycle (Ct) was defined as the fractional cycle number at which the fluorescence passed the fixed threshold. Each sample was measured in triplicate, and the relative amount of miR-124 to U6 was calculated using the $2^{-\Delta\Delta C_T}$ method.

Statistical analysis

All statistical analyses were performed with the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and graphs were generated via GraphPad Prism 5.0 (GraphPad Software Inc., USA). Data are shown as the mean $\pm$ standard deviation (SD). The Independent Student *t* test was used to compare the differences between groups. The relationship of miR-124 expression with clinicopathological factors was analyzed using Chi-square test. Patients survival and their differences were determined by the Kaplan-Meier method and log-rank test. A Cox's regression model was used for univariate and multivariate analysis. $P < 0.05$ was considered to indicated a statistically significant result.

Results

Aberrant expression of miR-124 in CCA tissues

Firstly, we compared the miR-124 expression in 119 CCA tissues and para-cancerous tissues with qRT-PCR assay. As indicated in **Figure 1**, the miR-124 level was significantly lower in CCA tissues than para-cancerous samples ($P < 0.001$), suggesting that expression of miR-124 was down-regulated in CCA tissues.

Association of miR-124 expression with clinicopathological characteristics in CCA

The correlation between miR-124 expression and clinicopathological parameters was examined in 119 cases (**Table 2**). Based on the median value (1.48) of miR-124 expression, all patients were divided into two groups as followings: low miR-124 expression group ($n=63$) and high miR-124 expression group ($n=56$). Among the parameters listed, a significant correlation was observed between low miR-124 expression and differentiation ($P=0.045$), lymph node metastasis ($P=0.040$). However, there were no relationships between miR-124 expression and other clinicopathological factors, including age, gender, tumor size, location, distant metastasis and TNM stage (all $P > 0.05$)(**Figure 2**).

Relationship between miR-124 expression and overall survival

Next, we analyzed the correlation of miR-124 expression with patients clinical outcome. Kaplan-Meier analysis showed that miR-124 expression was significantly associated with poor overall survival in CCA patients. According to log-rank test, the overall survival rate of patients with low miR-124 expression was lower than that of patients with high miR-124 expression ($P=0.001$).

In univariate Cox regression analysis, miR-124 expression ($P=0.003$, HR=2.107, 95%CI: 1.287-3.448) was a prognostic factor for cancer mortality in CCA. Additionally, lymph node metastasis ($P=0.001$, HR=2.269, 95%CI: 1.416-3.638) was also significantly related to overall survival. In a multivariate analysis, miR-124 expression was an unfavorable independent prognostic indicator in patients with CCA ($P=0.006$, HR=2.006, 95%CI: 1.224-3.289).

Discussion

CCA is the most common primary hepatic malignancy worldwide. Despite tremendous improvements in developing multimodal treatments, the clinical outcome of CCA patients remains unfavorable. Thus, it is of great significance to identify powerful prognostic indicator for CCA.

The discovery that non-coding components of the genome, including miRNAs, can conduct to the pathogenesis of cancer has led researchers to contemplate using these molecules to guide clinical strategies custom [16]. So far, a large number of studies suggest that miRNAs play a key role in tumor development. Increasing evidence suggest that deregulated expression of miRNAs participates in the initiation of CCA progression, where they act as either suppressors or promoters at the post-transcriptional regulation stage. For instance, Yang *et al.* reported that miR-144 was significantly down-regulated in CCA tissues and might be a tumor suppressor of CCA cell proliferation and invasion through targeting LIS1 [17]. Zhang *et al.* showed that miR-26a was increased in human CCA tissues and cell lines and overexpression of miR-26a increased proliferation of CCA cells and colony formation in *vitro* [18]. Wang *et al.* revealed that miR-138 was underexpressed in CCA tissues, and downregulation of miR-138 promoted the proliferation, migration and invasion of CCA cells [19]. Liu *et al.* confirmed that over-expression of miR-21 significantly promoted cell migration, invasion, and xenografts growth [20].

Previous research confirmed that miR-124 was dysregulated in various cancers, and aberrant miR-124 expression was involved in the prognosis of tumors. For example, Dong *et al.* showed that miR-124 had lower expression in breast cancer specimens and low miR-124 expression was a significant independent predictor of poor survival in breast cancer [21]. Gilje *et al.* showed that miR-124 is a potential new biomarker for prediction of neurological prognosis following cardiac arrest [22]. Han *et al.* demonstrated that miR-124 had a low expression in osteosarcoma tissues and low miR-124 expression was an unfavorable prognostic factor for overall survival [23]. Zhang *et al.* proved that miR-124 expression level was significantly decreased in lung cancer tissues and miR-124 was an independent prognostic factor for overall survival in lung cancer [24]. However, to our knowledge, the clinical significance of miR-124 in CCA has not been reported. Therefore, we investigated the feasibility of miR-124 as a new potential biomarker of prognosis for CCA.

In the present study, our data showed that miR-124 expression was significantly decreased in CCA tissues in comparisons with normal para-cancerous tissues. The relationship between miR-124 expression and various clinicopathological characteristics of CCA patients was investigated. The results indicated that abnormal miR-124 expression was dramatically correlated with differentiation and lymph node metastasis. It demonstrated that miR-124 might be involved in tumor metastasis and progression of CCA. Furthermore, the overall survival rate of low miR-124 group was significantly shorter than that of high miR-124 expression group. Finally, in a multivariate Cox model, we found that miR-124 expression was an independent poor prognostic indicator for overall survival of CCA patients. Our results were in line with previous studies. For example, Tian *et al.* showed that miR-124 was reduced in CCA tissues and negatively related to lymph node involvement and distant metastasis [25].

Conclusion

In conclusion, our results showed that miR-124 expression was remarkably reduced in CCA tissues. The expression of miR-124 was closely associated with the progression of CCA patients. More importantly, the down-regulation of miR-124 is an independent poor indicator of prognosis in CCA patients. We believed that it is just the beginning of a long and still unexplored avenue of a highly promising investigation of miR-124. A more in-depth and larger-scale study remains to identify the role of miR-124 in CCA.

Abbreviations

microRNAs (miRNAs)

cholangiocarcinoma (CCA)

quantitative reverse transcription polymerase chain reaction (qRT-PCR)

intrahepatic cholangiocarcinomas (IHCCs)

extrahepatic cholangiocarcinoma (EHCCs)

3'-untranslated region (3'-UTR)

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.

Consent for publication

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

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

Funding information is not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

N.W. design of the work; Y.L. the acquisition, analysis, Y.Z. interpretation of data; H.C. the creation of new software used in the work; X.W., Z.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Patel T: Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 2001, 33(6):1353-1357.
2. Bergquist A, von Seth E: Epidemiology of cholangiocarcinoma. *Best practice & research Clinical gastroenterology* 2015, 29(2):221-232.
3. Gatto M, Bragazzi MC, Semeraro R, Napoli C, Gentile R, Torrice A, Gaudio E, Alvaro D: Cholangiocarcinoma: update and future perspectives. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2010, 42(4):253-260.
4. Blehacz B, Gores GJ: Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008, 48(1):308-321.
5. Patel T: Cholangiocarcinoma—controversies and challenges. *Nature reviews Gastroenterology & hepatology* 2011, 8(4):189-200.
6. He L, Hannon GJ: MicroRNAs: small RNAs with a big role in gene regulation. *Nature reviews Genetics* 2004, 5(7):522-531.
7. Bartel DP: MicroRNAs: target recognition and regulatory functions. *Cell* 2009, 136(2):215-233.
8. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004, 116(2):281-297.
9. Hezova R, Kovarikova A, Srovnal J, Zemanova M, Harustiak T, Ehrmann J, Hajduch M, Sachlova M, Svoboda M, Slaby O: MiR-205 functions as a tumor suppressor in adenocarcinoma and an oncogene in squamous cell carcinoma of esophagus. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2016, 37(6):8007-8018.
10. Shenouda SK, Alahari SK: MicroRNA function in cancer: oncogene or a tumor suppressor? *Cancer metastasis reviews* 2009, 28(3-4):369-378.
11. Wang W, Luo YP: MicroRNAs in breast cancer: oncogene and tumor suppressors with clinical potential. *Journal of Zhejiang University Science B* 2015, 16(1):18-31.
12. Lee MR, Kim JS, Kim KS: miR-124a is important for migratory cell fate transition during gastrulation of human embryonic stem cells. *Stem Cells* 2010, 28(9):1550-1559.

13. Cheng LC, Pastrana E, Tavazoie M, Doetsch F: miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nature neuroscience* 2009, 12(4):399-408.
14. Zhang J, Lu Y, Yue X, Li H, Luo X, Wang Y, Wang K, Wan J: MiR-124 suppresses growth of human colorectal cancer by inhibiting STAT3. *PLoS one* 2013, 8(8):e70300.
15. Li L, Luo J, Wang B, Wang D, Xie X, Yuan L, Guo J, Xi S, Gao J, Lin X *et al*: Microrna-124 targets flotillin-1 to regulate proliferation and migration in breast cancer. *Molecular cancer* 2013, 12:163.
16. Nana-Sinkam SP, Croce CM: Clinical applications for microRNAs in cancer. *Clinical pharmacology and therapeutics* 2013, 93(1):98-104.
17. Yang R, Chen Y, Tang C, Li H, Wang B, Yan Q, Hu J, Zou S: MicroRNA-144 suppresses cholangiocarcinoma cell proliferation and invasion through targeting platelet activating factor acetylhydrolase isoform 1b. *BMC cancer* 2014, 14:917.
18. Zhang J, Han C, Wu T: MicroRNA-26a promotes cholangiocarcinoma growth by activating beta-catenin. *Gastroenterology* 2012, 143(1):246-256 e248.
19. Wang Q, Tang H, Yin S, Dong C: Downregulation of microRNA-138 enhances the proliferation, migration and invasion of cholangiocarcinoma cells through the upregulation of RhoC/p-ERK/MMP-2/MMP-9. *Oncology reports* 2013, 29(5):2046-2052.
20. Liu Z, Jin ZY, Liu CH, Xie F, Lin XS, Huang Q: MicroRNA-21 regulates biological behavior by inducing EMT in human cholangiocarcinoma. *International journal of clinical and experimental pathology* 2015, 8(5):4684-4694.
21. Dong LL, Chen LM, Wang WM, Zhang LM: Decreased expression of microRNA-124 is an independent unfavorable prognostic factor for patients with breast cancer. *Diagnostic pathology* 2015, 10:45.
22. Gilje P, Gidlof O, Rundgren M, Cronberg T, Al-Mashat M, Olde B, Friberg H, Erlinge D: The brain-enriched microRNA miR-124 in plasma predicts neurological outcome after cardiac arrest. *Crit Care* 2014, 18(2):R40.
23. Han G, Wang Y, Bi W, Jia J, Wang W: MicroRNA-124 functions as a tumor suppressor and indicates prognosis in human osteosarcoma. *Experimental and therapeutic medicine* 2015, 9(3):679-684.
24. Zhang Y, Li H, Han J: Down-regulation of microRNA-124 is correlated with tumor metastasis and poor prognosis in patients with lung cancer. *International journal of clinical and experimental pathology* 2015, 8(2):1967-1972.
25. Tian F, Chen J, Zheng S, Li D, Zhao X, Jiang P, Li J, Wang S: miR-124 targets GATA6 to suppress cholangiocarcinoma cell invasion and metastasis. *BMC cancer* 2017, 17(1):175.

Tables

Table 1. The relationship between miR-124 expression and clinicopathological features

Features	Cases (n=119)	MiR-124 expression		χ^2	P-value
		Low (n=63)	High (n=56)		
Age (years)					
<68	58	26	32	2.990	0.084
≥68	61	37	24		
Gender					
Male	73	43	30	2.695	0.101
Female	46	20	26		
Tumor size (cm)					
<5	63	33	32	0.271	0.603
≥5	54	30	24		
Location					
Intrahepatic	24	14	10	0.351	0.554
Extrahepatic	95	49	46		
Distant metastasis					
Negative	88	42	46	3.686	0.055
Positive	31	21	10		
Differentiation					
Well-moderate	76	35	41	4.006	0.045
Poor	43	28	15		
TNM stage					
I-II	61	28	33	2.489	0.115
III-IV	58	35	23		
Lymph node metastasis					
Absent	69	31	38	4.233	0.040
Present	50	32	18		

Table 2. The univariate and multivariate analyses of clinical factors for overall survival of CCA

Factors	Univariate analysis		Multivariate analysis	
	HR (95%CI)	<i>P</i> -value	HR (95%CI)	<i>P</i> -value
MiR-124 expression	2.107 (1.287-3.448)	0.003	2.006 (1.224-3.289)	0.006
Age	0.901 (0.565-1.438)	0.663	-	-
Gender	1.504 (0.924-2.448)	0.100	-	-
Tumor size (cm)	1.353 (0.848-2.160)	0.204	-	-
Location	0.814 (0.466-1.422)	0.470	-	-
Distant metastasis	1.397 (0.839-2.327)	0.199	-	-
Differentiation	1.153 (0.711-1.870)	0.563	-	-
TNM stage	1.446 (0.905-2.310)	0.122	-	-
Lymph node metastasis	2.269 (1.416-3.638)	0.001	2.175 (1.354-3.492)	0.001

Figures

Figure 1

The relative expression of miR-124 was examined in CCA tissues via qRT-PCR assay. Results showed that miR-124 level was significantly lower in CCA tissues compared with normal para-carcinoma tissues ($P<0.001$).

Figure 2

Kaplan-meier analysis of the impact of miR-124 expression on overall survival. Patients with low miR-124 expression had a shorter overall survival rate than those with high miR-124 expression ($P=0.002$).