

Received: 2016.03.27
Accepted: 2016.06.07
Published: 2016.07.11

miR-1260b is a Potential Prognostic Biomarker in Colorectal Cancer

Authors' Contribution:
Study Design A
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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: This work was supported by grants from the Natural Science Foundation of Gansu Province (number 145RJZA229)

Background: Colorectal cancer (CRC) mainly refers to colon and rectum cancer, which is the most common gastrointestinal malignant tumor. MicroRNAs (miRNAs) in tumors participate in multiple processes of malignancy development, including cell differentiation, proliferation, invasion, and metastasis. In this study we explored the relationship of miR-1260b abnormal expression with clinical pathological features in CRC patients.

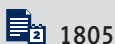
Material/Methods: The expression of miR-1260b was detected by real-time quantitative polymerase chain reaction (real-time PCR) in 120 cases of CRC tissues. The correlation of miR-1260b expression with the clinicopathologic features of CRC was analyzed by SPSS 21.0 statistical software. The Kaplan-Meier method was used for survival analysis. Cox regression analyses were conducted to determine whether miR-1260b was an independent predictor of survival for CRC patients.

Results: The miR-1260b expression in CRC was significantly higher than the expression levels in the corresponding para-carcinoma tissues ($P < 0.001$). According to the expression levels of miR-1260b, 120 cases of CRC patients were classified into either the miR-1260b high expression group or the miR-1260b low expression group. The high expression levels of miR-1260b in CRC patients was associated with lymph node metastasis ($P < 0.05$) and venous invasion ($P < 0.001$). However, the high miR-1260b expression had no significant correlation with other clinical parameters ($P > 0.05$). The high miR-1260b expression patients survived for shorter times than those CRC patients with low miR-1260b expression ($P < 0.05$). Multivariate analysis revealed that high miR-1260b means poor prognosis of patients with CRC.

Conclusions: The high expression level of miR-1260b is an independent prognostic biomarker that indicates a worse prognosis for patients with CRC.

MeSH Keywords: **Intestinal Neoplasms • MicroRNAs • Real-Time Polymerase Chain Reaction**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/898733>



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Background

Colorectal cancer (CRC) mainly refers to colon and rectum cancer, which is the most common gastrointestinal malignant tumor. The incidence and mortality rates of CRC are next only to those of gastric cancer, esophageal cancer, and primary liver cancer, which are all cancers that afflict the digestive system [1]. CRC patients at stage I-II and stage III have a better prognosis and five-year survival rate is 60–80%. However, prognosis of advanced CRC and postoperative recurrence of CRC is poor [2]. A large number of molecules and complicated signaling pathway regulations are involved in the occurrence and development of CRC, among which are oncogene activation, tumor suppressor gene inactivation, and epigenetic modifications [3]. Currently, research effort is being made to elucidate CRC pathogenesis, and there is an interest in further exploring the molecular dynamics and to target the molecular abnormalities in CRC.

MicroRNAs (miRNAs) are small RNAs (19–24 nucleotides in length) encoded by endogenous genes. miRNAs complementarily bind to the 3' untranslated regions (UTRs) of target genes to form the RNA-induced silencing complex (RISC), which results in the degradation of mRNA and inhibits the expression of target genes by translation or other regulatory mechanisms [4,5]. miRNAs can participate in many biological activities, including cell division, hormone secretion, and cancer development and progression [6]. More than 1000 kinds of human miRNA have been identified, and over 50% of the miRNA genes are located at susceptibility *loci* of tumor-associated chromosomes [7,8]. Compared with normal tissues, miRNA expression in tumor tissues is often significantly up-regulated or down-regulated. Such expression patterns act similarly to oncogenes or tumor suppressor genes by regulating the

downstream tumor-associated genes. Thus, miRNAs in tumors participate in multiple processes of malignancy development, including cell differentiation, proliferation, invasion, and metastasis [9,10]. By reviewing the relevant literature, we found that miR-1260b is abnormally expressed in multiple tumors, indicating that it may be a carcinogenic or tumor-suppressive miRNA. At present, the expression of miR-1260b in CRC is unclear. In the present study we investigated the expression levels of miR-1260b in CRC tissues and para-carcinoma tissues by real-time quantitative polymerase chain reaction (real-time PCR). We also explored the relationship of miR-1260b abnormal expression with clinical pathological features in CRC patients.

Material and Methods

Patients and samples

CRC tissues and adjacent tissues were obtained from 120 patients who had undergone complete surgical resection at the First Hospital of Lanzhou University from January 2009 to October 2010. All patients were confirmed for CRC by pathology, and they did not receive chemotherapy, radiotherapy, or immunotherapy before surgery. According to the TNM staging system developed by the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC), clinical staging for patients was performed. Specifically, 34 cases were in the I stage, 55 cases were in the II stage, and 31 cases were in the III stage. In this study, all patients with complete clinicopathological data signed an informed consent form. This investigation was approved by the in-house Medical Ethics Committee of the First Hospital of Lanzhou University.

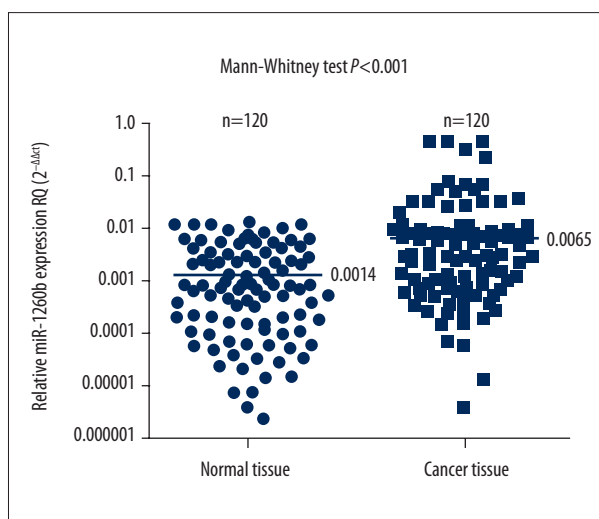


Figure 1. Expression level of miR-1260b in colorectal cancer and normal tissues.

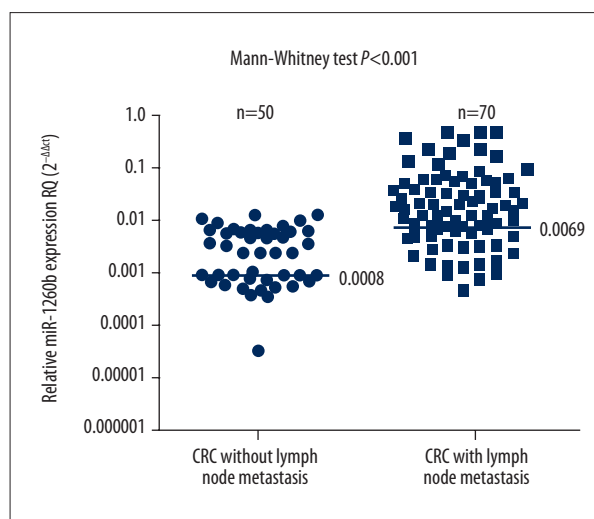


Figure 2. Expression level of miR-1260b in colorectal cancer tissues with and without lymph node metastasis.

Table 1. Correlation between miR-1260b expression and clinicopathological factors of colorectal cancer.

Variable	miR-1260b expression		P value
	Low (n=53)	High (n=67)	
Age (yr)			0.437
≤60	33	37	
>60	20	30	
Gender			0.880
Male	30	37	
Female	23	30	
Tumor size			0.888
≤5 cm	26	32	
>5 cm	27	35	
Degree of differentiation			0.509
Well and moderate	35	48	
Poor	18	19	
Venous invasion			<0.001
Negative	50	45	
Positive	3	22	
Depth of invasion			0.091
T1, T2	22	18	
T3, T4	31	49	
Lymph node metastasis			0.027
Absent	28	22	
Present	25	45	
Location			0.821
Colon	25	33	
Rectum	28	34	

Real-time PCR

CRC tissues and adjacent tissues were extracted for miR-1260b. Trizol (Invitrogen, CA) was used to extract the total RNA and was conducted as per the manufacturer's instructions. Total RNA isolated from patient samples were eluted in 50 µL diethyl pyrocarbonate (DEPC) water and stored at -80°C until use. The total RNA concentration was measured by ultraviolet spectrophotometry. Reverse transcription was carried out using reverse transcription kits (RR037A, TaKaRa Company, Japan) with 0.5 µg RNA, totaling to a final volume of 20 µL. Other reagents included: 5 µL 5×PrimeScript Buffer, 1 µL PrimeScript RT Enzyme Mix I (Haoqin Biotechnology, Shanghai, China), 2 µL stem-loop RT primer, and DEPC water. Reverse transcription reactions were as follows: 42°C for 15 min, 85°C for 5 s, and

4°C for 15 min, and then the reactions were stored at -80°C until further use for real-time PCR.

Real-time PCR reagents included: 10 µL Real-time PCR Master Mix, 1 µL upstream primer, 1 µL downstream primer, 2 µL cDNA, and DEPC water to bring to a total volume of 20 µL. Real-time PCR was performed using the 7300 Real-Time PCR System (Applied Biosystems) under the following conditions: 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. Real-time PCR of patient samples were repeated 3 times. The expression levels of miR-1260b was calculated by the comparative cycle threshold (Ct) method, and U6 small nuclear RNA was used as an internal reference. The relative expression of miR-1260b to U6 was determined using the equation $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = Ct_{miR-1260b} - Ct_{U6}$ [11].

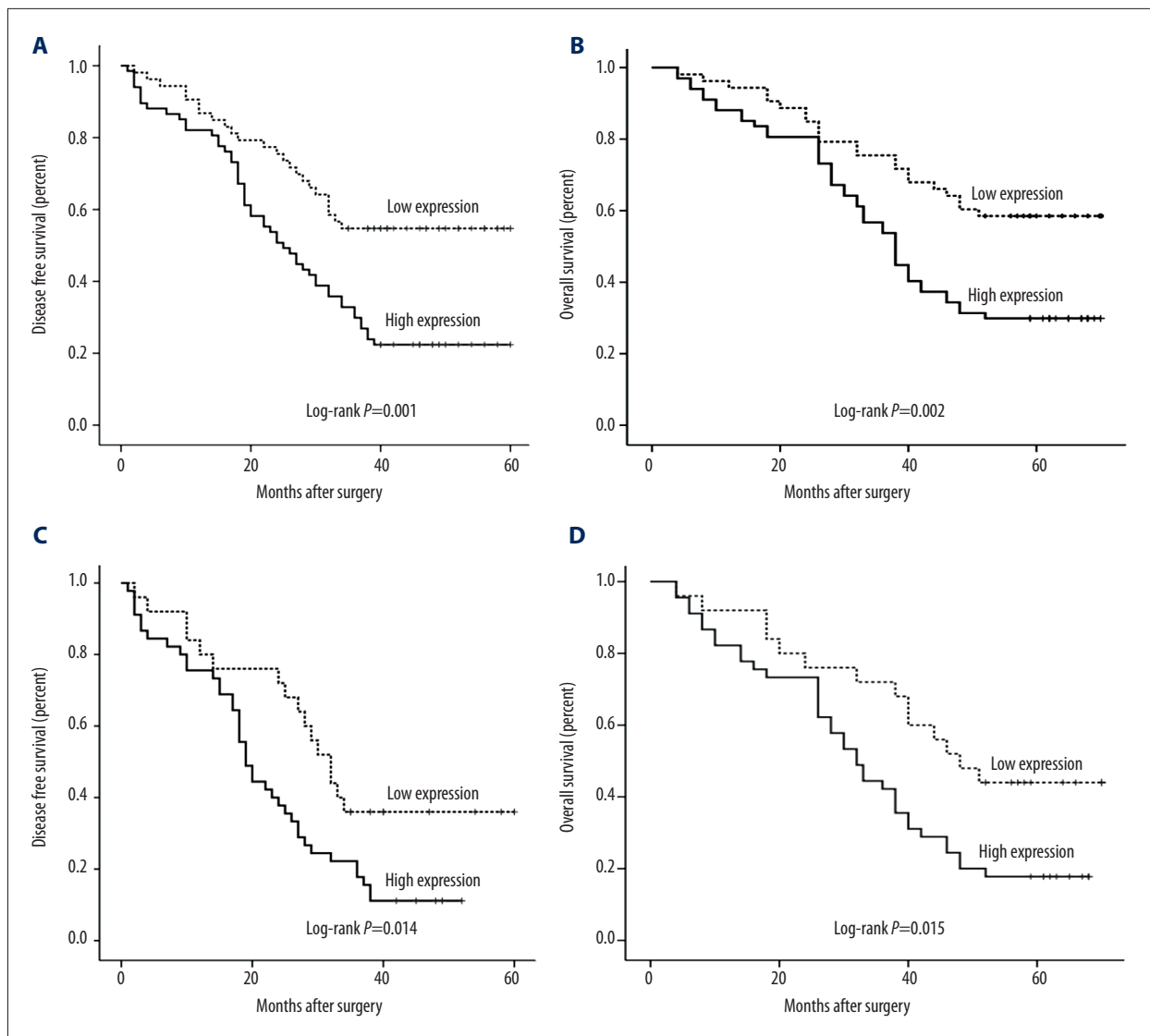


Figure 3. Kaplan-Meier survival curves of patients with colorectal cancer based on miR-1260b expression. **(A)** Disease-free survival of patients with colorectal cancer. **(B)** Overall survival of patients with colorectal cancer. **(C)** Disease-free survival of patients with lymph node metastases. **(D)** Overall survival of patients with lymph node metastases.

Statistical methods

We compared the miR-1260b expression in CRC cancer tissues with miR-1260b expression in para-carcinoma tissues or lymph node by paired t-test or Mann-Whitney test. Patients were divided into the miR-1260b low expression group and miR-1260b high expression group based on boundary values (median $2^{-\Delta\Delta Ct}$ values). The chi-square test was used for miR-1260b expression and clinicopathological parameters. The Kaplan-Meier method was used for survival analysis, and the log-rank test was used to compare patient survival between the 2 miR-1260b expression groups. To further analyze the survival data, the Cox proportional hazards model was used for joint effect analysis of each covariate. The overall survival

(OS) of CRC patients was considered as the primary outcome, and disease-free survival (DFS) was defined as the secondary outcome. Multivariate analysis was conducted using the Cox proportional hazards regression model. SPSS 21.0 statistical software (SPSS, Chicago, IL) was used for statistical analysis. P value of <0.05 was regarded as statistically significant.

Results

miR-1260b and CRC

Of all patients, 67 were male and 53 were female. The age range was 37–78 years old (average 53.6 ± 10.2 years old). Among the

Table 2. Univariate analysis of clinicopathological factors for overall survival of colorectal cancer patients.

Variable	Cases	HR	95%CI	P value
Age (yr)			0.604–1.571	0.913
≤60	70	1		
>60	50	0.974		
Gender			0.752–1.936	0.436
Male	67	1		
Female	53	1.207		
Tumor size			0.595–1.530	0.847
≤5 cm	58	1		
>5 cm	62	0.954		
Degree of differentiation			0.607–1.673	0.976
Well and moderate	83	1		
Poor	37	1.008		
Venous invasion			1.021–2.901	0.041
Negative	95	1		
Positive	25	1.721		
Depth of invasion			2.351–9.060	<0.001
T1, T2	40	1		
T3, T4	80	4.615		
Lymph node metastasis			1.626–4.778	<0.001
Absent	50	1		
Present	70	2.787		
Location			0.707–1.822	0.600
Colon	58	1		
Rectum	62	1.135		
miR-1260b expression			1.304–3.607	0.003
Low	53	1		
High	67	2.169		

120 patients in this study, there were 58 cases of colon cancer and 62 cases of rectal cancer. The miR-1260b expression in CRC was significantly higher than the expression levels in the corresponding para-carcinoma tissues ($P<0.001$, Figure 1). To explore the relationship of miR-1260b expression level with lymph node status, the expression level of miR-1260b in patients with different lymph node status was analyzed. Compared with patients without lymph node metastasis, the miR-1260b expression levels increased significantly in CRC tissues with lymph node metastasis ($P<0.001$) (Figure 2).

Expression of miR-1260b and clinicopathological features of CRC

According to the expression levels of miR-1260b ($2^{-\Delta\Delta Ct}$ median value as the cut-off value), 120 cases of CRC patients were classified into either the miR-1260b high expression group or the miR-1260b low expression group. Specifically, 67 patients with CRC were in the high expression group, and the other 53 were in the low expression group. We analyzed the relationship between clinicopathological features of CRC patients and miR-1260b expression (Table 1). The high expression levels of miR-1260b in CRC patients was associated with lymph node metastasis ($P<0.05$) and venous invasion ($P<0.001$). However, the high miR-1260b expression had no significant correlation with other clinical parameters, including age, sex, tumor

Table 3. Multivariate analysis of clinicopathological factors for overall survival of colorectal cancer patients.

Variable	Cases	HR	95%CI	P value
Venous invasion			0.630–2.011	0.690
Negative	95	1		
Positive	25	1.125		
Depth of invasion			2.138–8.759	<0.001
T1, T2	40	1		
T3, T4	80	4.327		
Lymph node metastasis			1.074–3.486	0.028
Absent	50	1		
Present	70	1.935		
miR-1260b expression			1.191–3.781	0.011
Low	53	1		
High	67	2.122		

size, degree of differentiation, depth of invasion, or tumor location ($P>0.05$).

miR-1260b expression and CRC patient prognosis

In order to analyze the relationship of miR-1260b expression levels between and prognosis of patient with CRCs, we plotted the survival curves (DFS and OS) of all patients by Kaplan-Meier method. The results suggested that the CRC patients with high miR-1260b expression levels had significantly shorter survival rates than low miR-1260b expression patients. (Figure 3). Univariate analysis showed that venous invasion ($P<0.05$), depth of invasion ($P<0.001$), lymph node metastasis ($P<0.001$), and high miR-1260b expression levels ($P=0.003$) were significantly correlated with OS (Table 2). Multivariate analysis using the Cox regression model suggested that high miR-1260b expression was an independent indicator of poor patient prognosis (Table 3).

Discussion

At present there are few reports about miR-1260b and there is no published research on the correlation between CRC and miR-1260b. Earlier studies showed that miR-1260b was abnormally and highly expressed in human peripheral blood dendritic cells, which suggests it may play an important role in the immune response of dendritic cells [12]. Stocklin et al. reported that miR-1260b may be associated with gum disease [13]. In addition, miR-1260b is a molecular marker with high sensitivity and specificity. Hirata et al. [14] determined that miR-1260b was highly expressed in renal cell carcinoma; they found that miR-1260b promoted renal cancer cell proliferation and invasion in renal cell carcinoma cells and genistein inhibited

Wnt-signaling by regulating miR-1260b expression in renal cancer cells. Another study reported that genistein exerts its anti-tumor effect via downregulation of miR-1260b, which target sRRP1 and Smad4 genes in prostate cancer cells [15]. The expression level of miR-1260b in non-small cell lung cancer tissues was higher than that of adjacent tissues, which might be related to the development of non-small cell lung cancer [16]. Therefore, miR-1260b may be involved in a variety of cellular activities, which provides a new way of thinking and direction for the study of CRC.

The existing research shows many miRNAs promote or inhibit the development of CRC through a variety of ways, and that miRNAs have phase specificity during tumor development [17,18]. Particularly, the same tumor has different miRNA expression profiles in different stages of development [19,20]. In a recent study, miRNAs were routinely monitored to predict onset of metastasis in breast cancer. Madhavan et al. identified panels of miRNAs, which include the metastasis-promoting miRNAs family, as well as oncogenic and tumor-suppressive miRNAs, which can serve as prognostic markers for malignant breast tumors. These studies provide us with a new way explore the panel of multiple miRNAs as prognostic factor of CRC [21]. In our study, we discovered that the expression levels of miR-1260b increased in CRC tissues compared with para-carcinoma tissues, and the miR-1260b expression was also up-regulated in CRC tissues with positive lymph nodes. We also evaluated the relationship between the expression level of miR-1260b and clinicopathological features of patients with CRC. The results showed that high miR-1260b expression was closely related with lymph node metastasis and venous invasion, which means that miR-1260b promotes the early-stage metastasis of CRC. In addition, we plotted the curves of DFS and OS using the Kaplan-Meier method, showing that higher

expression level of miR-1260b was closely related with poor DFS and OS, suggesting that miR-1260b may be a poor marker for prognosis of patients with CRC. Moreover, we used Cox regression analysis to evaluate some clinical and pathological factors. The final results suggest that high miR-1260b expression is an independent indicator of poor patient prognosis.

Conclusions

The high expression of miR-1260b suggests poor prognosis in patients with CRC. The results from this study conclude that

miR-1260b may be an important molecular marker for the prognosis of CRC. However, more rigorous clinical and basic studies are needed to confirm the present results.

Conflicts of interest

The authors report no conflicts of interest in this work.

Acknowledgement

We thank all the people and patients who participated in this study

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