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Original Article

Clinical significance and prognostic value of microRNA-23b expression level in colon cancer

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Abstract: Background: The clinical significance and prognostic value of miR-23b have not been investigated in colon cancer. Methods: qRT-PCR was used to determine the expression level of miR-23b in human colon cancer tissue samples. The association of miR-23b expression with clinicopathologic variables was analyzed. Kaplan-Meier survival analysis was performed to analyze the association of miR-23b expression with overall survival of patients. Univariate and multivariate Cox regression analyses were performed. Results: The result of qRT-PCR showed that the tissue expression levels of miR-23b were significantly down-regulated in colon cancer samples compared with those in adjacent normal tissues (1.672 ± 0.824 vs. 3.348 ± 1.327; P<0.001). Low miR-23b expression was observed to be significantly associated with advanced tumor stage (P<0.001), higher incidence of lymph node metastasis (P=0.004), higher incidence of distant metastasis (P=0.002), and tumor differentiation status (P<0.001). Kaplan-Meier analysis showed that the shorter overall survival time was significantly correlated with the low expression level of miR-23b (log-rank test: P=0.006). Furthermore, multivariate analysis with Cox's proportional hazards model confirmed that low miR-23b expression level was an independent predictor of poor prognosis for the colon cancer patients (Hazard ration=3.467; 95% CI: 1.455-15.864, P=0.005). Conclusion: This study indicated that down-regulation of miR-23b was associated with tumor progression and poor prognosis in colon cancer and was identified for the first time as an independent poor prognostic factor for colon cancer patients in Chinese population.

Keywords: microRNA, microRNA-23b, colon cancer, quantitative RT-PCR, prognosis

Introduction

Colon cancer is one of the most common, lethal diseases worldwide [1]. In China, colon cancer has become the fifth malignancy and its incidence has shown an obvious increasing trend over the decade [2]. Colon cancer patients with early stage can be treated successfully with surgical resection, and are most likely to be completely curative. However, for patients with advanced stage, tumor metastasis and chemotherapy resistance are the main reasons for its poor prognosis and high fatality rate [3]. Therefore, it is of great interest to search for valuable factors for early diagnosis for patients with a high risk of metastasis, prognosis prediction and novel therapeutic strategies.

MicroRNAs (miRNAs) are small non-coding RNAs of 20-22 nucleotides. It represses gene expression through interaction with 3'-untranslated regions (3'-UTRs) of mRNAs. miRNAs are predicted to target over 50% of all human protein-coding genes, enabling them to have numerous regulatory roles in many physiological and developmental processes, including development, differentiation, apoptosis and proliferation, through imperfect pairing with target mRNAs of protein-coding genes and the transcriptional or post-transcriptional regulation of their expression. Many miRNAs are deregulated in cancer. They are involved in tumorigenesis and function as oncogenes or tumor suppressor genes [4, 5].

MiRNA-23b (miR-23b), belonging to the miR-23b~27b~24-1 cluster (9q22.32), has been described as a pleiotropic modulator in different organs especially with regard to cancer development [6]. Previously, Zhang et al found that miR-23b was down-regulated in human colon cancer samples, and could potently

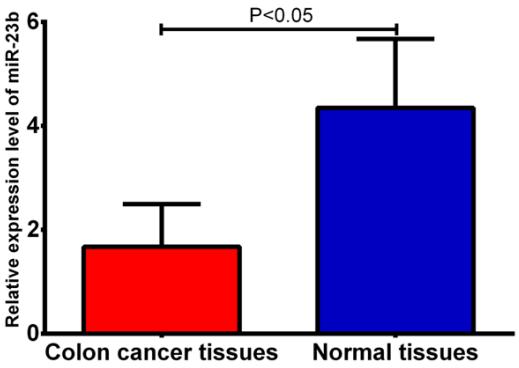

Figure 1. qRT-PCR detection of relative miR-23b expression in colon cancer tissues and adjacent normal tissues.

repress cancer cell migration, invasion, growth and angiogenesis both in vitro and in vivo. It directly regulated a cohort of prometastatic genes or oncogenes, including FZD7, MAP3K1, PAK2, TGFβR2, RRAS2 or uPA. Re-expression of these individual targets largely reversed miR-23-imposed invasion, whereas siRNA silencing each target impaired metastasis-relevant traits [7]. However, until now, the clinical significance and prognostic value of miR-23b in colon cancer have not been investigated.

Materials and methods

Patients and samples

This study consisted of 110 patients with colon cancer. All patients were recruited at the department of gastrointestinal surgery, affiliated Yongchuan hospital of Chongqing medical university from March 2008 to December 2015. One hundred and ten pairs of surgical specimens of colon cancer tissues and adjacent normal tissues were collected and immediately placed in liquid nitrogen and then stored at -80°C until the isolation of RNA. Clinicopathological data including age, gender, location, tumor size, pathological stage, and tumor differentiation were collected. Overall survival time was defined as the time from surgical resection to date of last follow-up or death. The diagnosis of all specimens was histopathologically confirmed by two pathologists and staging was determined according to the 7th edition of cancer staging criteria of the American Joint Committee On Cancer (AJCC). The study was approved by the Research Ethics Committee of the Affiliated Yongchuan Hospital of Chongqing Medical University. Written informed consent was obtained from all of the patients.

RNA isolation and qRT-PCR

Total RNA was extracted from colon cancer tissues and matched normal adjacent tissues by homogenizing tissue in Trizol reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. Primers for miR-23b and endogenous control U6 snRNA were obtained from Applied Biosystems (Foster City, California, USA). The concentration and purity of RNA were determined spectrophotometrically using the NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, Delaware, USA). cDNA was generated using the PrimeScript RT reagent kit (Takara Co. Ltd, Dalian, China) in a 20 μl final reaction volume containing 0.5 μg of RNA, 0.5 μl Prime-Script RT enzyme mix, and 4 μl 5× PrimeScript buffer, and 1 μl RT primer, and incubated at 42°C for 60 min and at 85°C for 5 min. Quantitative real-time PCR assay was performed to evaluate miR-23b expression using SYBR Premix Ex Taq (Takara Co. Ltd) and measured in a LightCycler 480 System (Roche, Basel, Switzerland). The amplification profile was denatured at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. Relative quantification of miRNA expression was performed using the $2^{-\Delta\Delta CT}$. The raw data were presented as the relative quantity of target miRNA, normalized with respect to U6 snRNA and relative to a calibrator sample.

Statistical analysis

The comparison of the expression level of miR-23b between colon cancer tissues and adjacent normal tissues was performed using the two-sample Student's t test. The correlation between the expression of miR-23b and clinicopathological characteristics was assessed with the two-sample Student's t test. The survival rate was computed by the Kaplan-Meier method, and the differences of survival rate between groups were evaluated with the log-rank test. Multivariate analyses were conducted for the risk factors for colon cancer according to the Cox's proportional hazards model. All calculations were performed with the SPSS 18.0 statistical software package (SPSS, Inc., Chicago, IL). *P* values<0.05 were considered as significant.

Table 1. Relation between miR-23b expression and clinical characteristics in patients with colon cancer

Characteristics	Cases	Tissue miR-23b level		*P* value
		Low (n=55)	High (n=55)	
Age (years)				
≥55	43	24	19	0.435
<55	67	31	36	
Gender				
Female	37	19	18	0.500
Male	73	36	37	
Smoking status				
No	21	8	13	0.332
Yes	89	47	42	
Tumor size (cm)				
≥3	63	34	29	0.441
<3	47	21	26	
Lymph node metastasis				
No	75	30	45	0.004
Yes	35	25	10	
Distant metastasis				
No	91	39	52	0.002
Yes	19	16	3	
Histological subtypes				
Adenocarcinoma	66	37	29	0.173
Squamous cell carcinoma	44	18	26	
Differentiation status				
Well	32	6	26	<0.001
Moderate/poor	78	49	29	
Clinical stage				
I-II	69	23	46	<0.001
III-IV	41	32	9	

Results

Expression level of miR-23b and correlations with clinicopathological characteristics in colon cancer

The result of qRT-PCR showed that the tissue expression levels of miR-23b were significantly down-regulated in colon cancer samples compared with those in adjacent normal tissues (1.672 ± 0.824 vs. 3.348 ± 1.327; P<0.001, shown in **Figure 1**). After normalization to RNU6B expression levels, the median level of miR-23b in colon cancer tissues was used as a cutoff point to divide all 110 patients into two groups: low miR-23b group (n=55, patients who expressed miR-23b at levels less than the cut-off value) and high miR-23b group (n=55, patients who expressed miR-23b at levels more than the cutoff value). **Table 1** summarized the association between miR-23b expression and clinicopathologic variables in colon cancer patients. By statistical analysis, low miR-23b expression was observed to be significantly associated with advanced tumor stage (P<0.001), higher incidence of lymph node metastasis (P=0.004), higher incidence of distant metastasis (P=0.002), and tumor differentiation status (P<0.001). However, there were no significant difference between miR-23b expression and other variables of patients including gender (P=0.500), age (P=0.435), smoking status (P=0.332), tumor size (P=0.441), and histological subtypes (P=0.173).

Relationship between miR-23b expression level and survival time

Totally, 110 patients were included for survival analysis. The miR-23b expression level was classified as high or low in relation to the median value. Next, the overall survival curves of colon cancer were charted using the Kaplan-Meier method. The results showed that the shorter overall survival time of colon cancer patients was significantly correlated with the low expression level of miR-23b (log-rank test: P=0.006, shown in **Figure 2**). The multivariate analysis with Cox's proportional hazards model confirmed that low miR-23b expression level was an independent predictor of poor prognosis for the colon cancer patients (Hazard ration=3.467; 95% CI: 1.455-15.864, P=0.005, shown in **Table 2**).

Discussion

The development of colon cancer is a complex process that requires a series of integrated steps including cellular neoplastic transformation, unlimited growth, and the acquisition of invasive/metastatic properties, as well as immunologic escape. Although extensive investigation explored some important factors of colon cancer, the effect of various treatment approaches including surgical operation, chemotherapy and immune cell based therapy remains limited because of the complex process of development of colon cancer [8, 9].

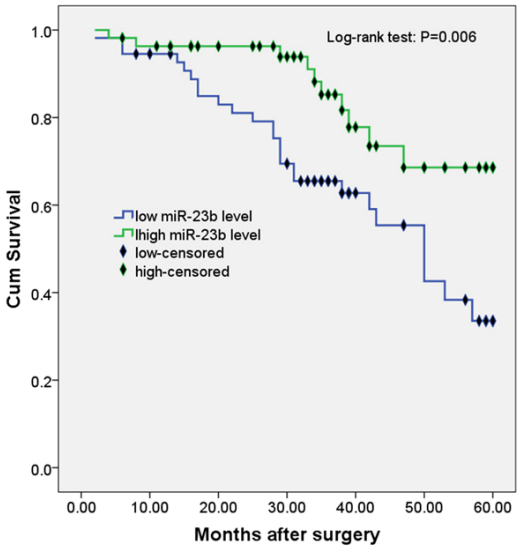

Figure 2. Kaplan-Meier curve for overall survival in colon cancer patient with low versus high miR-23b expression.

Therefore, the goal to attain a more thorough understanding of the molecular biology, genetic causes, and cellular origin of colon cancer is of great significance in the development of improved therapeutic strategies and in the identification of prognostic markers.

Tumorigenesis is a process that results from genetic lesions and epigenetic changes. Searching for alterations in the colon cancer genome and identifying novel cancer-related genes is a focus for research for the treatment of cancer. Recently, researchers have begun to understand that miRNAs are also involved in cancer-related processes in humans, such as the regulation of cancer proliferation, differentiation, angiogenesis, apoptosis, and metastatic potential [10]. miRNAs function as negative endogenous gene-expression regulators by binding complementary sequences in target mRNAs, resulting in their selective degradation or selective steric inhibition of translation [11]. Therefore, miRNAs are involved in a wide range of biological functions [12]. Aberrant miRNA expression has been reported to be involved in the pathogenesis of Alzheimer's disease, cardiovascular disease, spinal motor neuron anomalies, and numerous others [13]. Furthermore, deregulated expression of miRNAs has been identified in a variety of human malignancies, suggesting that miRNAs function as potential oncogenic factors or tumor suppressors, depending on the cell type or tissue investigated and their target genes [14]. For example, the miR-34 family members are direct transcriptional targets of p53 and their expression induces cell cycle arrest in cancer cell lines [15]. miRNA-29 family members act as tumor suppressors through the restoration of a normal DNA methylation pattern [16].

It has been determined that miR-23b mediates the various steps in the metastatic process, including tumor growth, invasion, and even angiogenesis by repressing a cohort of prometastatic targets [7]. The concrete role of miR-23b in carcinogenesis is complex and might depend on cancer types. Majid et al. showed that miR-23b played a tumor-suppressive role in prostate cancer by repressing Src kinase expression [17]. However, miR-23b can act as an oncogene in renal carcinoma via downregulating the expression level of proline oxidase [18]. The clinical significance of miR-23b has been investigated in some types of cancer. For example, Janikova et al found that miR-23b was mostly downregulated in NSCLC samples and that its upregulation in tumors was connected with longer progression-free survival (P=0.065) and overall survival (P=0.048), indicating that miR-23b might serve as a suitable prognostic biomarker for NSCLC patients [19]. The study by Zhuang et al indicated that plasma miR-23b was overexpressed in gastric cancer patients and high plasma miR-23b expression was associated with poor clinical outcome. Thus, plasma miR-23b might serve as a potential diagnostic biomarker and therapeutic target for gastric cancer [20]. Li et al found that the down-regulation of miR-23b might function as tumor suppressor through inhibiting the upregulation of RUNX2 in ovarian cancer and it was significantly correlated with tumor aggressiveness and poor prognosis of patients with ovarian cancer, indicating it might be a potential prognostic marker for ovarian cancer [21].

The expression level and function of miR-23b have been investigated in colon cancer. Previously, Zhang et al found that miR-23b was down-regulated in human colon cancer samples, and could potently repress cancer cell migration, invasion, growth and angiogenesis both in vitro and in vivo. It directly regulated a cohort of prometastatic genes or oncogenes, including FZD7, MAP3K1, PAK2, TGFβR2, RRAS2 or uPA. Re-expression of these individu-

Table 2. Multivariate analysis for overall survival in 110 patients with colon cancer

Factor	Parameter	Hazard ratio	95% CI	*P* value
Age	≥55	1.945	0.653-4.925	0.221
	<55			
Gender	Female	0.672	0.281-1.991	0.573
	Male			
Smoking status	No	2.187	0.572-2.334	0.314
	Yes			
Tumor size (cm)	≥3	2.765	0.879-4.882	0.083
	<3			
Lymph node metastasis	Yes	3.231	2.129-12.338	0.003
	No			
Distant metastasis	Yes	4.542	3.191-19.002	<0.001
	No			
Histological subtypes	Adenocarcinoma	0.892	0.376-2.034	0.733
	Squamous cell carcinoma			
Differentiation status	Moderate/poor	3.441	1.287-8.882	0.0125
	Well			
Clinical stage	III-IV	4.862	2.011-18.921	<0.001
	I-II			
miR-23b level	Low	3.467	1.455-15.864	0.005
	High			

al targets largely reversed miR-23-imposed invasion, whereas siRNA silencing each target impaired metastasis-relevant traits [7]. However, until now, the clinical significance and prognostic value of miR-23b in colon cancer have not been investigated. In the present study, qRT-PCR showed that the tissue expression levels of miR-23b were significantly down-regulated in colon cancer samples compared with those in adjacent normal tissues. By statistical analysis, low miR-23b expression was observed to be significantly associated with advanced tumor stage, higher incidence of lymph node metastasis, higher incidence of distant metastasis, and tumor differentiation status, suggesting that miR-23b was associated with the occurrence, progression, and metastasis of colon cancer. Then the overall survival curve of colon cancer was charted using the Kaplan-Meier method. The results showed that the shorter overall survival time of colon cancer patients was significantly correlated with the low expression level of miR-23b. Furthermore, the multivariate analysis with Cox's proportional hazards model confirmed that low miR-23b expression level was an independent predictor of poor prognosis for the colon cancer patients.

In conclusion, this study indicated that down-regulation of miR-23b was associated with tumor progression and poor prognosis in colon cancer and was identified for the first time as an independent poor prognostic factor for colon cancer patients in Chinese population.

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Disclosure of conflict of interest

None.

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References

[1] Siegel R, Desantis C and Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin 2014; 64: 104-117.

[2] Wan DS. [Epidemiologic trend of and strategies for colorectal cancer]. Ai Zheng 2009; 28: 897-902.

[3] Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J and Bouvier AM. Epidemiology and management of liver metastases from colorectal cancer. Ann Surg 2006; 244: 254-259.

[4] van Kouwenhove M, Kedde M and Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. Nat Rev Cancer 2011; 11: 644-656.

[5] Calin GA and Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.
[6] Donadelli M and Palmieri M. Roles for microRNA 23b in regulating autophagy and development of pancreatic adenocarcinoma. Gastroenterology 2013; 145: 936-938.
[7] Zhang H, Hao Y, Yang J, Zhou Y, Li J, Yin S, Sun C, Ma M, Huang Y and Xi JJ. Genome-wide functional screening of miR-23b as a pleiotropic modulator suppressing cancer metastasis. Nat Commun 2011; 2: 554.
[8] Mannucci S, Ghin L, Conti G, Tambalo S, Lascialfari A, Orlando T, Benati D, Bernardi P, Betterle N, Bassi R, Marzola P and Sbarbati A. Magnetic nanoparticles from Magnetospirillum gryphiswaldense increase the efficacy of thermotherapy in a model of colon carcinoma. PLoS One 2014; 9: e108959.
[9] Ciardiello F, Kim N, Saeki T, Dono R, Persico MG, Plowman GD, Garrigues J, Radke S, Todaro GJ and Salomon DS. Differential expression of epidermal growth factor-related proteins in human colorectal tumors. Proc Natl Acad Sci U S A 1991; 88: 7792-7796.
[10] Esquela-Kerscher A and Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.
[11] Caldas C and Brenton JD. Sizing up miRNAs as cancer genes. Nat Med 2005; 11: 712-714.
[12] Iorio MV and Croce CM. MicroRNAs in cancer: small molecules with a huge impact. J Clin Oncol 2009; 27: 5848-5856.
[13] Haramati S, Chapnik E, Sztainberg Y, Eilam R, Zwang R, Gershoni N, McGlinn E, Heiser PW, Wills AM, Wirguin I, Rubin LL, Misawa H, Tabin CJ, Brown R Jr, Chen A and Hornstein E. miRNA malfunction causes spinal motor neuron disease. Proc Natl Acad Sci U S A 2010; 107: 13111-13116.
[14] Lee HW, Lee EH, Ha SY, Lee CH, Chang HK, Chang S, Kwon KY, Hwang IS, Roh MS and Seo JW. Altered expression of microRNA miR-21, miR-155, and let-7a and their roles in pulmonary neuroendocrine tumors. Pathol Int 2012; 62: 583-591.
[15] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA and Hannon GJ. A microRNA component of the p53 tumour suppressor network. Nature 2007; 447: 1130-1134.
[16] Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K and Croce CM. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci U S A 2007; 104: 15805-15810.
[17] Majid S, Dar AA, Saini S, Arora S, Shahryari V, Zaman MS, Chang I, Yamamura S, Tanaka Y, Deng G and Dahiya R. miR-23b represses proto-oncogene Src kinase and functions as methylation-silenced tumor suppressor with diagnostic and prognostic significance in prostate cancer. Cancer Res 2012; 72: 6435-6446.
[18] Liu W, Zabirnyk O, Wang H, Shiao YH, Nickerson ML, Khalil S, Anderson LM, Perantoni AO and Phang JM. miR-23b targets proline oxidase, a novel tumor suppressor protein in renal cancer. Oncogene 2010; 29: 4914-4924.
[19] Janikova M, Zizkova V, Skarda J, Kharaishvili G, Radova L and Kolar Z. Prognostic significance of miR-23b in combination with P-gp, MRP and LRP/MVP expression in non-small cell lung cancer. Neoplasma 2016; 63: 576-587.
[20] Zhuang K, Han K, Tang H, Yin X, Zhang J, Zhang X and Zhang L. Up-Regulation of Plasma miR-23b is Associated with Poor Prognosis of Gastric Cancer. Med Sci Monit 2016; 22: 356-361.
[21] Li W, Liu Z, Chen L, Zhou L and Yao Y. MicroRNA-23b is an independent prognostic marker and suppresses ovarian cancer progression by targeting runt-related transcription factor-2. FEBS Lett 2014; 588: 1608-1615.