

The expression and Clinical Significance of miRNA-135a and Bach1 in colorectal cancer

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Abstract

AIM To explore the correlation between the expression of miRNA-135a and Bach1 in colorectal cancer tissue and the patient's clinical information. **Methods** 60 patients with colorectal carcinoma were treated as a control group. Real-time quantitative PCR assays and immunohistochemistry method were performed to detect the expression of miRNA-135a and Bach1 in 60 colorectal carcinoma and adjacent normal tissues, and the clinical and pathological classifications had also been investigated. The SPSS 19.00 software was used. All data represent mean±SD of three independent experiments. $P < 0.05$ was considered statistically significant. **Results** miRNA-135a expression levels increased significantly in the colon cancer tissues compared with the non-tumor control tissues ($P < 0.01$). miRNA-135a expression levels were higher in stage III/IV than in stage I/II colon cancer patients. The expression level of Bach1 in colorectal cancer was significantly lower ($P < 0.01$). Bach1 and miRNA-135a were negatively correlated. **Conclusions:** The levels of miRNA-135a and Bach1 were opposite, the over-expression of miRNA-135a might decrease Bach1, which may be involved in the pathogenesis of colorectal cancer.

Background

The colorectal cancer (CC) mortality rate has been decreasing in Western advanced countries, while is still growing in China. Recently, CC has become the third-ranking cause of cancer death in China. During the early stages of CC, some patients could be treated effectively with radical surgery and chemotherapy. Due to the high rates of postsurgical recurrence and metastasis, the prognosis remains disappointing for patients with advanced-stage^[1-3].

MicroRNAs (miRs), a category of non-protein-coding RNAs, have been recognized as critical participants in many pathways, especially proliferation and apoptosis. Besides, more and more researches have displayed their carcinogenic or cancer suppressive functions in many solid tumors^[4-7]. Recently, miR-135a has been explored widely and deeply because of its controversial role in cancers^[8-10]. For example, the expression of miR-135a increases in hepatocellular carcinoma and human bladder cancer, which is implicated in the development of them. By contrast, some studies show that it decreases and plays a suppressive role during the development of malignant glioma, such as epithelial ovarian cancer and renal cell carcinoma^[11-14]. These controversial results may reflect the various roles of miR-135a in different types of cancer. Furthermore, miR-135a has been found to be up-regulated in CC cells^[9,15]. As one of potential target genes, Bach1 (BTB and CNC homology 1), plays a vital role in adjustment of oxidative stress and ascribed as a repressor of its main target hemoxygenase-1 (HO-1). The expression of HO-1 increases significantly in various types of cancer, which might promote tumor growth and metastasis^[16-18]. In this study, we examined the expression levels of miR-135a and Bach1 in CC tissues by quantitative PCR and immunohistochemistry respectively, and investigated the association between them to evaluate the possible role in the development of CC.

Methods

Tissue samples and clinical data

Sixty patients diagnosed with CC at Wuxi People's Hospital of Nanjing Medical University between 2016 and 2017 were recruited in our study. These patients were treated by colectomy with lymphadenectomy. The clinical stage of postoperative patients was evaluated. All patients should not received any chemotherapy, radiotherapy or other treatment prior to surgery. Human tissues including sixty colorectal cancer tissues and sixty matched adjacent normal tissues were immediately collected after surgical resection. The clinicopathologic characteristics of these patients were collected from electronic medical records. The study was approved by the Research Ethics Board of Wuxi People's Hospital of Nanjing Medical University. All patients signed an informed consent form for this investigation.

quantitative PCR

Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, California) and then measured by spectrophotometer (BioPhotometer, Eppendorf, Hamburg, German).

The transcriptions of miRNA-135a and Bach1 were detected with the primers. The transcription of b-actin was used for normalization. The PCR products were detected by ethidium bromide staining. Images were obtained and the gray values of all the products were measured by ImageJ.

Immunohistochemistry

Immunohistochemical study was performed using the EnVision method (Dako, Glostrup, Danmark) on 2-mm formalin-fixed, paraffin-embedded sections. The staining intensity was scored semiquantitatively as described by two independent observers without knowledge of the clinical status of the samples^[14]. All the images were captured using a digital camera mounted on a light microscope (Axioscopic, Zeiss, Gottingen, Germany).

Statistical analyses

Data presented as the mean±SD and analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Statistical analyses were performed using an independent samples t-test and one-way ANOVA. Spearman correlation analysis was performed. P<0.05 was considered significant.

Results

The clinicopathologic characteristics of these patients showed in Table 1. There was no difference between the expression of miRNA-135a with many aspects in CC tissues, including age, tumor size, location, differentiation and etc. It was different in lymph node involvement group and tumor stage group. Compare to lymph node negative involvement group, miRNA-135a expression levels were higher in lymph node involvement group. Besides, miRNA-135a expression levels were higher in stage III/IV than in stage I/II colon cancer patients (P <0.01) (Fig 1). The results suggested that the high expression of miRNA-135a

in lymph node metastasis(LNM) group and tumor III-IV stages presented the potential correlation with LNM and tumor stage.

miRNA-135a had a statistical difference between the CC tissues and matched normal tissues ($P < 0.01$). The expression levels of miRNA-135a were significantly increased in CC tissues (figure 2).

The expression of Bach1 by RT-PCR result was 0.032 ± 0.002 vs 0.073 ± 0.004 $P < 0.05$, in the CC tissues and matched normal tissues, respectively, suggesting a significant decrease of Bach1 during the development of CC (Fig 3).

Compared with the normal tissues group, a significant decrease of Bach1 in CC tissues was also observed (Fig 4).

The expression of Bach1 was different in tumor stage group ($P < 0.01$) (table 2). The results suggested that the low expression of Bach1 in tumor III-IV stages presented the potential correlation with tumor stage.

miRNA-135a expression level was higher in stage III/IV colon cancer patients, while the expression level of Bach1 was significantly lower in the same stage. The levels of miRNA-135a and Bach1 were opposite and negatively correlated. ($P < 0.05$) (table 3).

Discussion

The morbidity incidence of CC has ranked no. 3 among all malignant tumor diseases in China. CC affects approximately 390,000 new patients in China annually, and the mortality has ranked no. 5 among all malignant tumors. Tumor metastasis is the major cause of death in CC patients. miRNAs are small non-coding RNAs, they induce their degradation or block the translation of the encoded protein via binding to specific complementary sequences in the 3'UTR of target mRNAs. With the diverse abilities, reducing of their expression has might been involved with promoting or suppressing tumor metastasis, providing a new perspective on the metastatic process. As is well-known, miRNAs could promote or inhibit various traits related to tumor aggressiveness such as proliferation, cell migration and invasion in various cancer cell lines. As one member of them, miRNA-135a can produce an identical and active sequence through being encoded by two genes localized on different chromosomes. Current reports have shown that the effects of miRNA-135a on cancer progression are contradictory. Previous researches showed that the expression of miRNA-135a decreased in human gastric cancer, the proliferation of gastric cancer cells was repressed while the apoptosis was promoted^[10]. On the other hand, miRNA-135a showed a inhibitive role during the migration and invasion of lung cancer cells, due to targeting a transcription factor^[19]. However, the functions and mechanisms of miRNA-135a during tumors are largely unknown^[20-23]. Recent studies have demonstrated that miRNA-135a is up-regulated in CC cell lines SW480 and SW620, while in our study, the expression levels of miRNA-135a were significantly increased in CC tissues, which was in accordance with previous studies. With the analysis of clinicopathologic characteristics, the expression levels of miRNA-135a were obviously different between different tumor

stage groups, and it was different in lymph node involvement group. They were positively related. The data also showed no correlation between miRNA-135a and many aspects in CC tissues, including age, tumor size, location, differentiation and etc. These results supported the hypothesis that miRNA-135a was involved in CC progression, which might function as an oncogenic factor.

Bach1, a member of the basic leucine zipper transcription factor family, is a critical participant in the process of oxidative stress^[24]. Recent researches demonstrate that Bach1 is a widely expressed transcriptional repressor, it takes part in many vital cell processes through the targeted genes, such as cell cycle progression, apoptosis, and the hypoxia response negatively^[25-28]. Heme-oxygenase-1 (HO-1), one of the target genes, might be significant in induction of the tumorigenic pathway. The significant increasing expression of HO-1 in various types of cancer is contributed to promote tumor growth and metastasis. Furthermore, Bach1 is recognized to inhibit growth and survival of acute myeloid leukemia (AML) cells by down-regulation of HO-1 expression. Bach1 functions as a repressor of HO-1 in human renal cancer cells, though the possible mechanism has not been established^[17,29]. In addition, the up-regulation of HO-1 might inhibit apoptosis of renal cancer cells via activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway.^[29,30] Our results show that the expression of Bach1 significantly decreased in CC tissues. With further analysis, there was no difference in tumor I-II stages while the majority decrease was in tumor III-IV stages, which was similar to miRNA-135a. These findings indicated that Bach1 might play an inhibition role during the development of CC, especially in the advanced stages. Additionally, Bach1 and miRNA-135a were negatively correlated. Thus, it could be concluded that miRNA-135a played an oncogenic role in CC through down-regulation of Bach1 at least partially. Bach1 might be one of targets of miRNA-135a. However, our study indicated the potential role of miRNA-135a and Bach1, further research should be needed to explore the exact signal pathway between them during the development of CC.

Conclusions

In summary, the results presented here indicated that the expression of miRNA-135a was activated significantly, which likely decreased the expression of Bach1 and involved in CC progression. Thus, the expression of miRNA-135a might be useful as a prognostic biomarker and a possible therapeutic target for CC patients.

Abbreviations

CC: colorectal cancer; miRs: MicroRNAs; HO-1: hemoxygenas-1; AML: acute myeloid leukemia; BACH1: BTB and CNC homology 1; PCR: Polymerase Chain Reaction; LNM: lymph node metastasis; Nrf2: nuclear factor (erythroid-derived 2)-like 2.

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Board of Wuxi People's Hospital of Nanjing Medical University. All patients signed an informed consent form for this investigation.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

ZF and TQ were responsible for overall study concept and design of experiments. WJ, ZC analyzed and interpreted the patient data. JY performed the IHC analysis and PCR, and ZF was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Correlations between clinicopathological parameters and miRNA-135a expression in CC tissues

Parameters	Patient	colorectal cancers miRNA-135a	Adjacent normal tissues		
			P-value	miRNA-135a	P-value
Age					
<60	26	0.086±0.050		0.041±0.003	
≥60	34	0.079±0.019	0.56	0.038±0.006	0.49
Gender					
Male	33	0.085±0.042		0.040±0.006	
Female	27	0.076±0.011	0.47	0.039±0.005	0.55
Tumor size					
<5 cm	31	0.086±0.044		0.042±0.005	
≥5 cm	29	0.077±0.012	0.46	0.037±0.003	0.45
Tumor location					
Ascending colon	8	0.085±0.049		0.040±0.005	
Transverse colon	3	0.084±0.043		0.038±0.002	
Descending colon	6	0.082±0.027		0.041±0.003	
Sigmoid colon	9	0.078±0.015		0.040±0.004	
Rectum	4	0.083±0.033	0.74	0.040±0.002	0.63
Differentiation					
Well and moderately	29	0.085±0.041		0.041±0.005	
Poorly	31	0.077±0.011	0.54	0.038±0.003	0.47
Depth of invasion					
T ₁ ~T ₂	22	0.087±0.049		0.039±0.004	
T ₃ ~T ₄	38	0.078±0.017	0.48	0.040±0.006	0.58
Lymph node involvement					
Negative	21	0.054±0.015		0.041±0.002	
Positive	39	0.100±0.028	0.01	0.039±0.005	0.49
Tumor stage					
I-II	26	0.062±0.021		0.040±0.005	
III-IV	34	0.103±0.032	0.01	0.039±0.006	0.52

Table 2 Expression of BACH1 in different tumor stage

stage	n	Bach1		P
		+	-	
I/II	26	10	16	
III/IV	34	3	31	0.05

The low expression of BACH1 in tumor III-IV stages presented the potential correlation with tumor stage.

Table 3 Correlation between miR-135a expression and BACH1 in CC tissues

		Bach1		R	P
		low	high		
miRNA-135a	low	8	5	-0.375	0.04
	high	41	6		

Bach1 and mirna-135a are negatively correlated.(P <0.05)

Figures

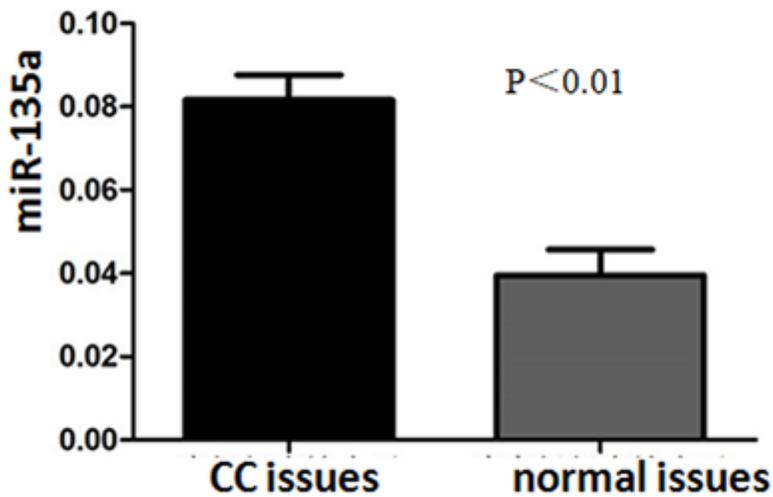


Figure 1

Expression of miR-135a in CC tissues

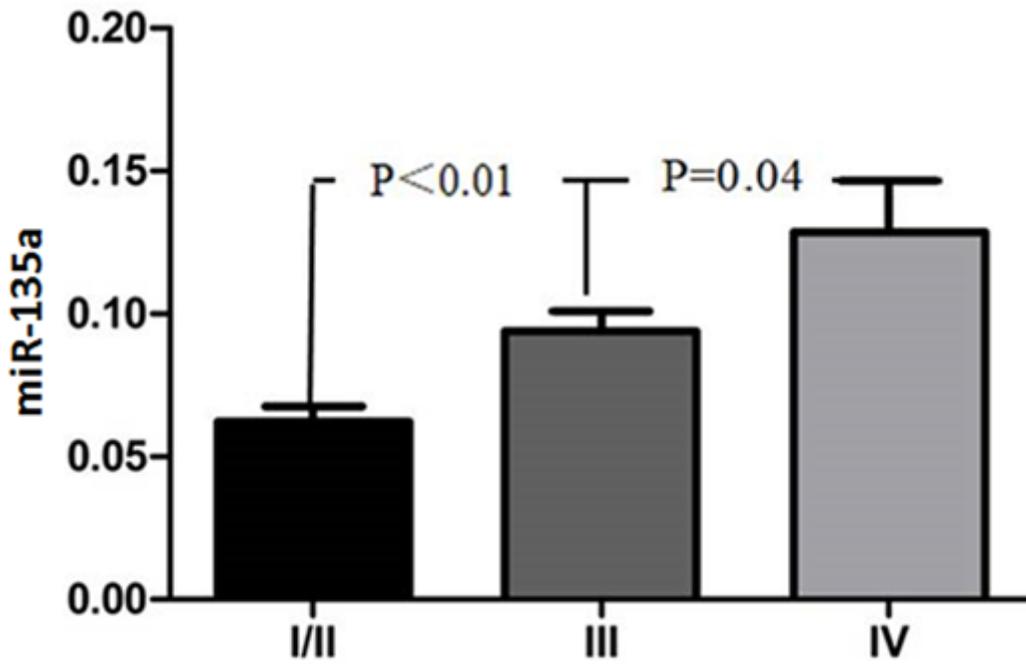


Figure 2

Expression of miR-135a in different tumor stage

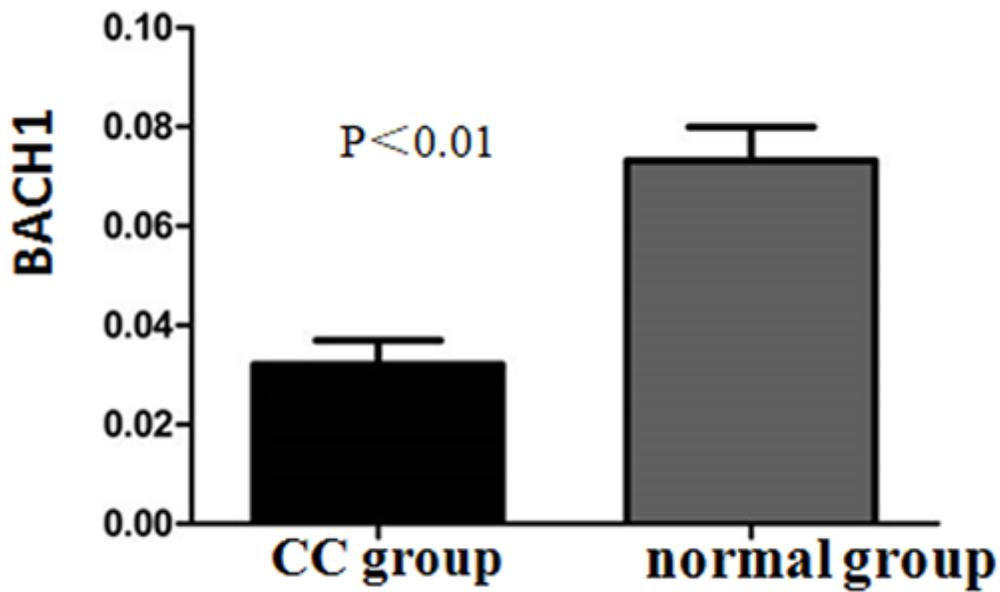


Figure 3

Expression of BACH1 in CC tissues

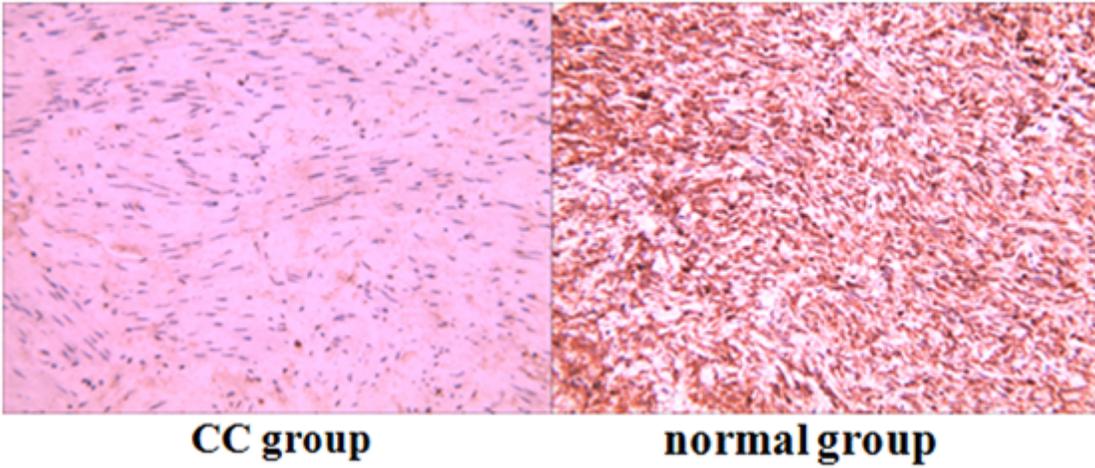


Figure 4

BACH1 expression evaluated by immunohistochemistry($\times 200$). Compare to normal group, the expression of BACH1 in CC issues was much smaller.