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

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Research article

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Abstract

BACKGROUND

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 50 colorectal carcinoma and adjacent normal tissues, and the clinical and pathological classifications have 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 increase significantly in the colon cancer tissues compared with the non-tumor control tissues(P<0.01). miRNA-135a expression levels are 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 are negatively correlated.

Conclusions: mirna-135a and bach1 showed a negative correlation, it may be one of the important indices for colorectal cancer screening.

Background

The colorectal cancer (CC) mortality rate has being decreasing in Western advanved 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 contributes to the development. By contrast, the expression of miR–135a 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 upregulated in CC cells[9,15]. As one of 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 hemoxygenas–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 level of miR–135a and BACH1 in CC tissues by quantitative PCR and

immunohistochemistry respectively, and investigated the association between miR–135a and BACH1 expression to evaluate the possible role in the development of CC.

Material And 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 colorectectomy 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 -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 formalinfixed, 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 (Axioscrop, Zeiss, Gottingen, Germany).

Statistical analyses

The results were provided as the mean±SD and analyzed using SPSS 19.0 software. Statistical analysis was performed using an independent samples ttest and one-way AVOVA. Spearman correlation analysis was performed. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The clinicopathologic characteristics of these patients showed at Table 1. The results showed that miR–135a were different in lymph node involvement group and tumor stage group. Compare to lymph node negative involvement group, miRNA–135a expression levels are higher in lymph node involvement group. Besides, miRNA–135a expression levels are 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 miR–135a in lymph node metastasis(LNM) group and tumor III-IV stages presented the potential correlation with LNM and tumor stage.

miR–135a had a statistical difference between the CC tissues and matched normal tissues (P < 0.01). The expression levels of miR–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±0.004P<0.05, in the CC tissues and matched normal tissues, respectively, suggesting a significant decrease of BACH1 with the development of colorectal cancer (Fig 3).

Compared with the normal tissues group, a significant decrease of BACH1 in CC tissues was also observed (Fig 4).

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

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

Discussion

Tumor metastasis is the major cause of death in CC patients. miRNAs are small non-coding RNAs, which bind to specific complementary sequences in the 3'UTR of target mRNAs and induce their degradation or block the translation of the encoded protein. This raises the possibility that an entire pathway/process may be controlled at several levels through a single miR. With the diverse abilities, reducing of their expression has might been associated with promoting or suppressing tumor metastasis, providing a new perspective on the metastatic process. miR–135a is encoded by two genes localized on different chromosomes, producing an identical and active sequence. Recent reports have shown that the effects of miR–135a on cancer progression are contradictory. 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. Previous researches showed that the expression of miR–135a decreased in human gastric cancer, the proliferation of gastric cancer cells was repressed while the apoptosis was promoted [10].On the other hand, miR–135a showed a inhibitive role during the migration and invasion of lung cancer cells by targeting a transcription factor [19].However, the functions and mechanisms of miR–135a

are largely unknown[20–23].Recent studies have demonstrated that miR–135a is upregulated in CC cell lines SW480 and SW620, while in our study, the expression levels of miR–135a were significantly increased in CC tissues, which was in accordance with previous studies. Besides, miR–135a were different in lymph node involvement group and tumor stage group, they were positively related. These results support the hypothesis that miR–135a is involved in CC progression, which may function as an oncogenic factor.

BACH1, a member of the basic leucine zipper transcription factor family, is a critical participant in the regulation of oxidative stress[24].Recent researches demonstrated that BACH1 is a widely expressed transcriptional repressor, which takes part in cell cycle progression, apoptosis, and the hypoxia response negatively through the targeted genes[25–28].As one of them, heme-oxygenase–1 (HO–1) might be significant in induction of the tumorigenic pathway. The expression of HO–1 increases significantly in various types of cancer, which is proved to promote tumor growth and metastasis and suppress the apoptosis of tumor cells. Furthermore, BACH1 is recognized to inhibit growth and survival of acute myeloid leukemia (AML) cells by downregulation of HO–1 expression. Despite of the mechanisms by which BACH1 plays a role in renal cancer development have not been established, there is a research showing that BACH1 functions as a repressor of HO–1 in human cells[17,29].In additional, the up-regulation of HO–1 might inhibits 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 with the development of colorectal cancer, these data combined with our findings indicate that BACH1 might play a inhibition role during the development of CC. Additionally, Bach1 and miR–135a were negatively correlated. Thus, BACH1 might be one of targets of miR–135a, miR–135a might play an oncogenic role in CC, at least partially, through down-regulation of BACH1. However, our study indicated the potential role of miR–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 indicate that the expression of miR–135a was activated significantly, which likely decreased the expression of BACH1 and involved in CC progression. Thus, the expression of miR–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 available from the corresponding author on reasonable request.

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Competing Interests

No potential conflicts of interest

Authors' contributions

Zhu YF and Tao GQ designed research; Wang J, Zhang YC, treated patients and collected material and clinical data from patients; Jiang ZY performed the assays; Zhang YC analyzed the data; Zhu YF wrote the paper.

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References

1. Brenner H, Kloor, M, Pox CP, et al. Colorectal cancer. Lancet 2014;**383**:1490-1502.
2. Saif MW, Chu E. Biology of colorectal cancer. Cancer J 2010, 16(3):196-201.
3. Yiu AJ, Yiu CY. Biomarkers in Colorectal Cancer. Anticancer Res,2016,36(3):1093-102.
4. Cekaite L, Eide PW, Lind GE, et al. microRNAs as growth regulators, their function and biomarker status in colorectal cancer.Oncotarget,2016,7(6):6476-6505.
5. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers.Nature.2005,435,834-838.
6. Hollis M, NairK, Vyas A, et al. MicroRNAs potential utility in colon cancer：Early detection, prognosis, and chemosensitivity. World J Gastroenterol,2015,21(27): 8284-8292.
7. Ng EK, Chong WW, Jin H, et al. Differential expression of MicroRNAs in plasma of patients With colorectal cancer：a potential marker for colorectal cancer screening. Gut, 2009,58(10):1375-1381.
8. Liang C, Sun W, He H, et al. Antitumor effect of a new nano-vector with miRNA-135a on malignant glioma. Int J Nanomedicine.2018 ;13:209-220.
9. Zhou W, Li X, Liu F, et al. MiR-135a promotes growth and invasion of colorectal cancer via metastasis suppressor 1 in vitro. Acta Biochim Biophys Sin (Shanghai).2012;44(10):838-46.
10. Liu L, Ye JX, Qin YZ, et al. Evaluation of miR-29C, miR-124, miR-135a and miR-148a in Predicting lymph node metastasis and tumor stage of Gastric Cancer. Int J Clin EXP Med,2015,8(12):22227-22236.
11. Mao XP, Zhang LS, Huang B, et al. Mir-135a enhances cellular proliferation through post-transcriptionally regulating PHLPP2 and FOXO1 in human bladder cancer. J Transl Med.2015 13;13:86.
12. Yamada Y, Hidaka H, Seki N, et al. Tumor-suppressive MicroRNA-135a inhibits cancer cell proliferation by targeting the c-MYC oncogene in renal cell carcinoma. Cancersci,2013,104(3):304-312.
13. Tang W, Jiang Y, Mu X, et al. MiR-135a functions as a tumor suppressor in epithelial ovarian cancer and regulates HOXA10 expression. Cell Signal.2014;26(7):1420-6.
14. Leung CO, Deng W, Ye TM, et al. miR-135a leads to cervical cancer cell transformation through regulation of β-catenin via a SIAH1-dependent ubiquitin proteosomal pathway. 2014;35(9):1931-40.
15. Schlörmann W，Naumann S，Renner C, et al. Influence of miRNA-106b and miRNA-135a on butyrate-regulated expression of p21 and Cyclin D2 in human colon adenoma cells. Genes Nutr.2015;10(6):50.
16. Davudian S, Mansoori B, Shajari N, et al. BACH1, the master regulator gene: A novel candidate target for cancer therapy.2016;588(1):30-7.

17. Chen Y, Zhang J, Wang H, et al. miRNA-135a promotes breast cancer cell migration and invasion by targeting HOXA10. BMC Cancer.2012 Mar 23 ;12:111
18. Kocanova S, Buytaert E, Matrouble JY, et al. Induction of heme-oxygenase 1 requires the p38MAPK and PI3K pathways and suppresses apoptotic cell death following hypericin-mediated photodynamic therapy. 2007;12(4):731-41.
19. Shi H, Ji Y, Zhang D, et al. MiR-135a inhibits migration and invasion and regulates EMT-related marker Genes BY targeting KLF8 in lung Cancer Cells. Biochem Biophys Res Commun,2015,465(1):125-130
20. Schlörmann W, Naumann S, Renner C, et al. Influence of miRNA-106b and miRNA-135a on butyrate-regulated expression of p21 and Cyclin D2 in human colon adenoma cells. Genes Nutr.2015;10(6):50
21. Ahmad A, Zhang W, Wu M, et al. Tumor-suppressive miRNA-135a inhibits breast cancer cell proliferation by targeting ELK1 and ELK3 oncogenes. Genes Genomics.2018;40(3):243-251.
22. Ren JW, Li ZJ, Tu C. MiR-135 post-transcriptionally regulates FOXO1 expression and promotes cell proliferation in human malignant melanoma cells. Int J Clin EXP Pathol,2015,8(6):6356-6366
23. Wang Q, Zhang H, Shen X, Ju S. Serum microRNA-135a-5p as an auxiliary diagnostic biomarker for colorectal cancer. Ann Clin Biochem,2017,54(1):76-85.
24. Davudian S, Mansoori B, Shajari N, et al. BACH1, the master regulator gene: A novel candidate target for cancer therapy. 2016;588(1):30-7
25. Anderson NM, Simon MC. BACH1 Orchestrates Lung Cancer Metastasis. 2019;178(2):265-7.
26. Lee J, Yesilkanal AE, Wynne JP, et al. Effective breast cancer combination therapy targeting BACH1 and mitochondrial metabolism. 2019 ;568(7751):254-8.
27. Shajari N, Davudian S, Kazemi T, et al. Silencing of BACH1 inhibits invasion and migration of prostate cancer cells by altering metastasisrelated gene expression. Artif Cells Nanomed Biotechnol.2018;46(7):1495-1504.
28. Davudian S, Shajari N, Kazemi T, et al. BACH1 silencing by siRNA inhibits migration of HT-29 colon cancer cells through reduction of metastasis-related genes. Biomed Pharmacother.2016;84:191-198.
29. Li S1, Chen T, Zhong Z, et al. microRNA-155 silencing inhibits proliferation and migration and induces apoptosis by upregulating BACH1 in renal cancer cells. Mol Med Rep.2012r;5(4):949-54.
30. . Chang LC, Fan CW, Tseng WK, et al. Immunohistochemical Study of the Nrf2 Pathway in Colorectal Cancer: Nrf2 Expression is Closely Correlated to Keap1 in the Tumor and Bach1 in the Normal Tissue. Appl Immunohistochem Mol Morphol.2013;21(6):511-7.

Tables

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

Parameters	Patient	□□miRNA-135a	*P*-value	□□miRNA-135a	Pvalue
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□+□	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

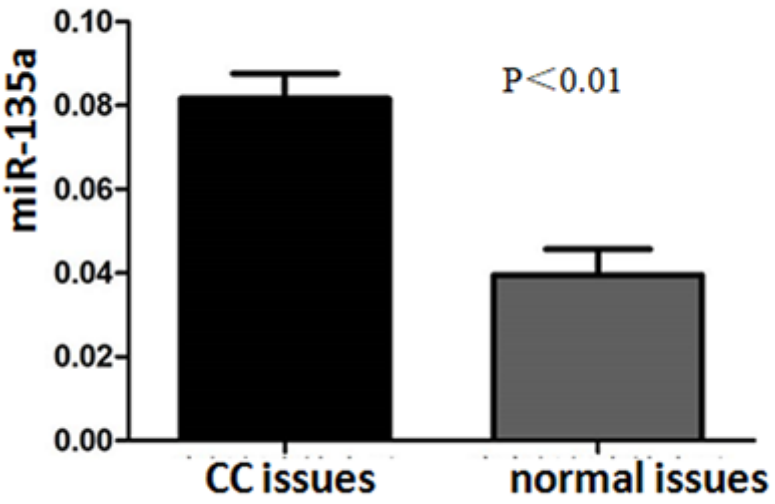

Figure 1

Expression of miR-135a in CC tissues

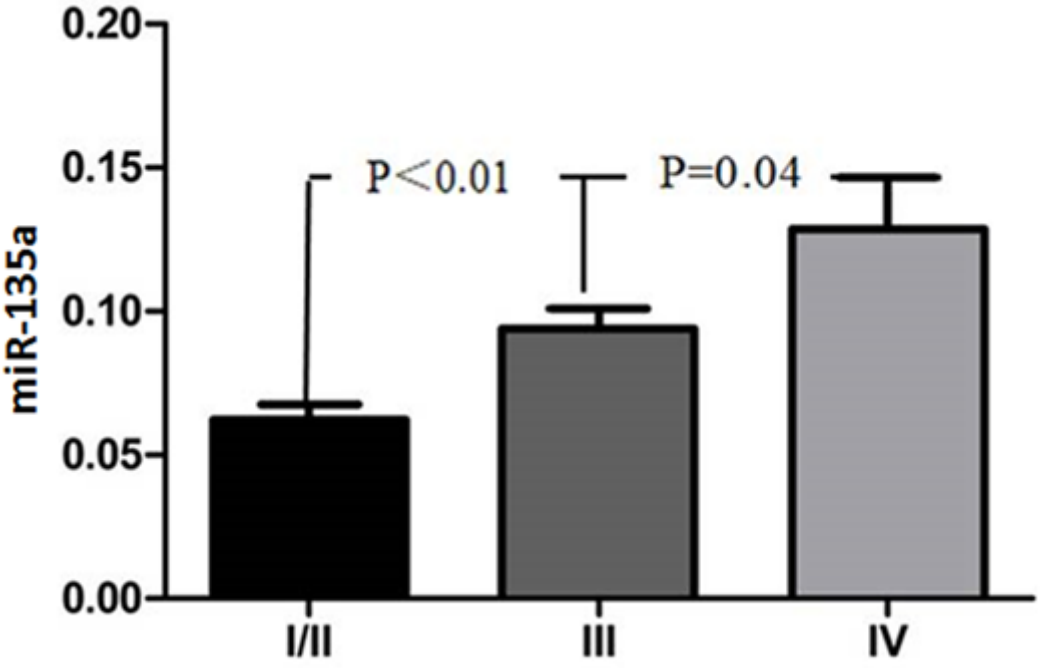

Figure 2

Expression of miR-135a in different tumor stage

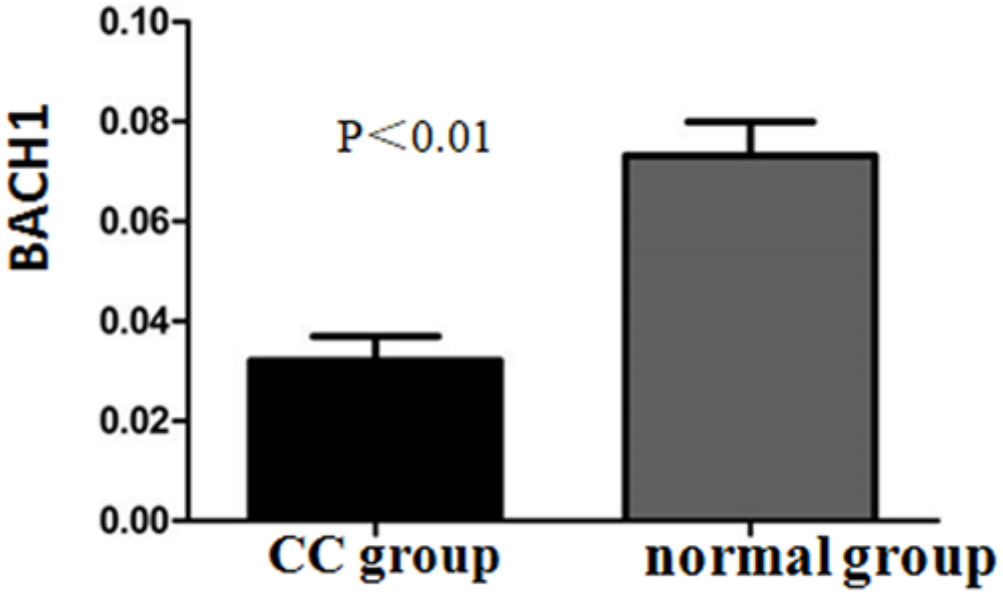

Figure 3

Expression of BACH1 in CC tissues

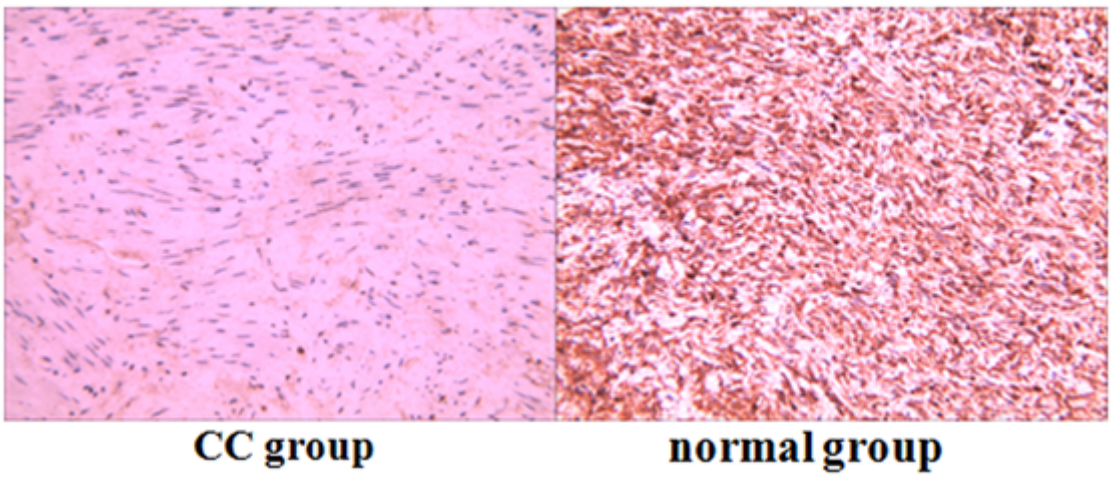

Figure 4

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