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Original Article

BC200 RNA is over-expressed in colorectal cancer and promotes migration and invasion of HCT116 cells

Peng Li[1,2*], Bo Yang[3*], Shaoyou Xia[2,4*], Li Chen[5], Ning Ning[2,4], Bing Ma[2,4], Qing Liu[2], Huaxia Yang[2], Ding Zhang[2], Xiaohui Du[2,4]

[1]Department of Oncology Surgery, The Chinese PLA General Hospital, Beijing 572013, China; [2]Department of General Surgery, Hainan Branch of The Chinese PLA General Hospital, Sanya, Hainan, China; [3]Department of General Thoracic Surgery, The Chinese PLA General Hospital, Beijing, China; [4]Department of General Surgery, The Chinese PLA General Hospital, Beijing, China; [5]The Emergency Department, The Chinese PLA General Hospital, Beijing, China. [*]Co-first authors.

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Abstract: Objective: To explore the expression of BC200 RNA in colorectal cancer and its possible function associated with cell proliferation, migration and invasion. Methods: BC200 RNA expression levels in the specimens of 36 patients with colorectal cancer (CRC) and in human CRC cell line HCT116 were assessed by real time PCR. HCT116 cells were transfected with BC200 siRNA or negative control siRNA. MTS assay was performed to evaluate the cell proliferation. Scratch wound healing assay and Matrigel™ invasion assay were applied to assess the migration and invasion abilities of HCT116. Results: The results showed that BC200 RNA expression was remarkably elevated in tumor tissues as compared with adjacent normal tissues, and was also positively correlated with EPCAM RNA expression. Knock-down of BC200 RNA expression by siRNA significantly inhibited the cell migration and invasion, but did not affect the cell proliferation evidently. Conclusions: This study firstly approves that BC200 RNA is overexpressed in CRC, and functionally associated with cell migration and invasion. BC200 RNA may be utilized as a novel molecular marker and therapeutic target of CRC.

Keywords: ncRNA, BC200, colorectal cancer, EPCAM, invasion

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors worldwide [1]. As well known, a complex gene interaction and molecular modulation network is involved in the carcinogenesis and progression of CRC.

Nowadays, more and more researches are focused on exploring the molecular modulation network and finding reliable diagnostic markers and effective therapeutic targets, which will be valuable for oncologists to make better treatment decisions in cancer clinic. In recent years, a growing number of non-coding RNAs have been found linked with various types of cancer [2-5]. And several lncRNAs have already been demonstrated to play crucial roles in the cancer origination and development in CRC [6-8].

BC200 (BCYRN1) is a non-coding RNA with the length of 200 nt, which is an important translational regulator and prevalently expressed in the nervous system [9]. The expression of BC200 RNA is also found deregulated in a variety of carcinomas, such as breast cancer, lung cancer, cervix cancer and ovarian cancer, etc. [10]. For instance, in breast cancer, BC200 RNA has been identified as an important indicator of tumor invasion [11]. However, the expression pattern and regulating role of BC200 RNA in CRC has not been reported yet. BC200 RNA transcript is located at 2q21, and adjacent to the EPCAM promoter. EPCAM is an oncogene that plays a pivotal role in cell migration and invasion, and also a predictor of outcome in cancer patients [12, 13]. It has been reported that the ncRNAs and their adjacent coding genes, especially those ncRNAs located at the upstream side of the coding genes promoters [14], just like BC200 and EPCAM, are often functionally associated with each other. So, we speculated that the expression of BC200 RNA may be correlated with that of EPCAM, and the function of BC200 RNA may be linked with the cell migration and invasion in CRC, just like EPCAM.

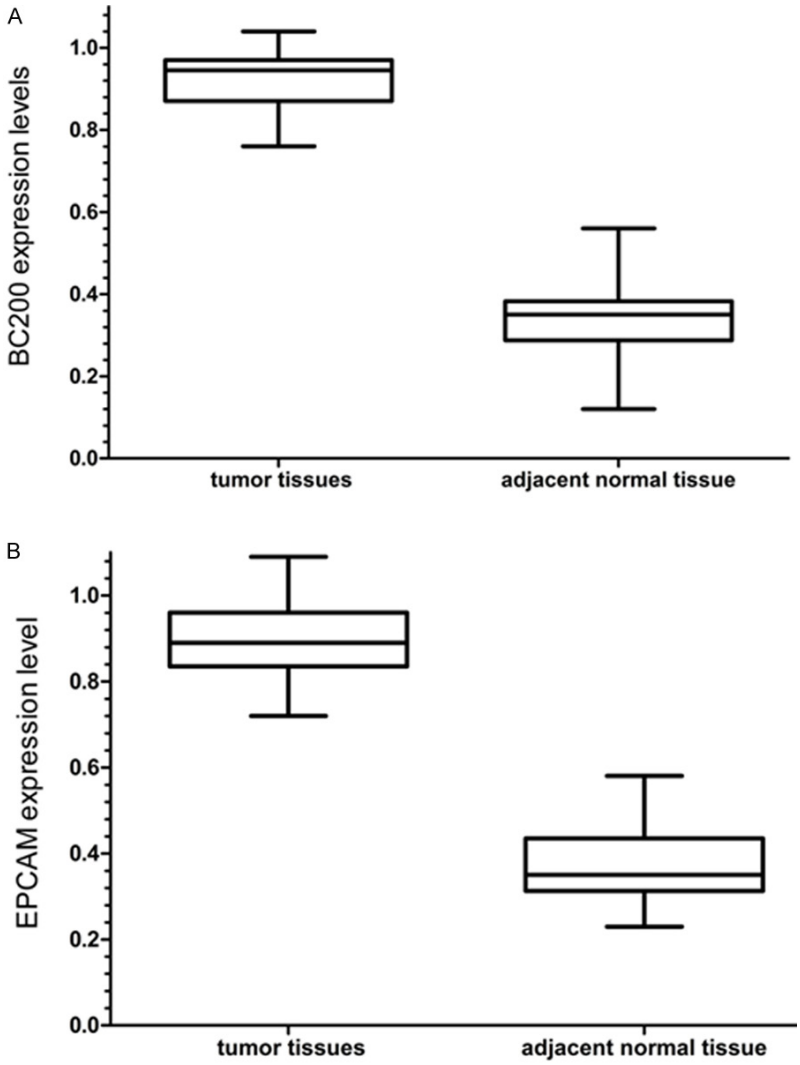

Figure 1. A, B. The BC200 and EPCAM expression levels were analyzed by real time PCR in 36 CRC tissue samples.

Therefore, in this study, we investigated the expression levels of BC200 RNA in 36 cases patients with CRC, both in the tumor tissues and adjacent normal tissues. Meanwhile, we also explored the correlation between the expression levels of BC200 RNA and that of EPCAM in these specimens. Moreover, we assessed the effect of BC200 knockdown on the in vitro growth characteristics of CRC cell line HCT116.

Materials and methods

Patient samples

Thirty-six cases of colorectal cancer (CRC) patients who accepted initial surgery in Chinese PLA General Hospital from 2009 to 2014 were retrospectively recruited for this study. No patient had received chemotherapy or radiation therapy before resection. The utilization of tumor material for research was approved by the ethical committee of Chinese PLA General Hospital.

Cancer cell lines

The human CRC cell line HCT116 was purchased from American Type Culture Collection (Manassas, VA, USA). The HCT116 cells were cultured in DMEM medium (Gibco, Carlsbad, CA) containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA) at 37°C with 5% CO_2.

Small interference RNA (siRNA) analysis

For small interfering RNA (siRNA) analysis, siRNA for BC200 RNA sequence, and non-targeting siRNA, were obtained from GenePharma (Shanghai, China). BC200 RNA: sense strand: 5'-GGAUAACAGGCAUGAGCCACCACAT-3', antisense strand: 5'-AUGUGGUGGCUCAUGCCUGUUAUCCCA-3'. Approximately 5% HCT116 cells were plated to each well of 12-well plates at least 24 h before transfection to achieve 70-80% confluency. SiRNA transfection was done with lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad CA) according to the manufacturer's instructions. Cells were collected after transfection for RNA isolation, MTS cell proliferation assay, scratch wound healing assay and Matrigel™ invasion assay.

Real-time quantitative PCR

Total RNA was isolated from CRC tumor tissue, matched adjacent normal tissue and CRC cells by using Trizol™ Total RNA Reagent (Invitrogen,

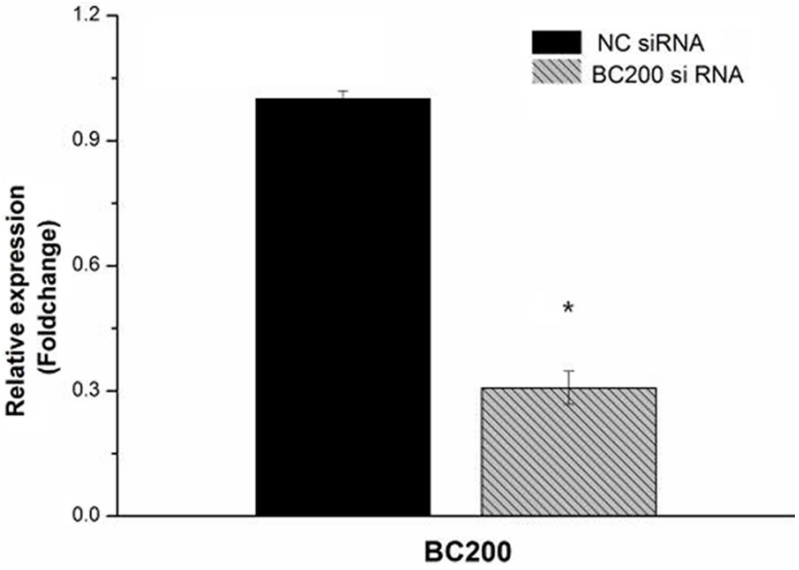

Figure 2. Expression of BC200 RNA in BC200 siRNA or negative control siRNA treated HCT116 cells.

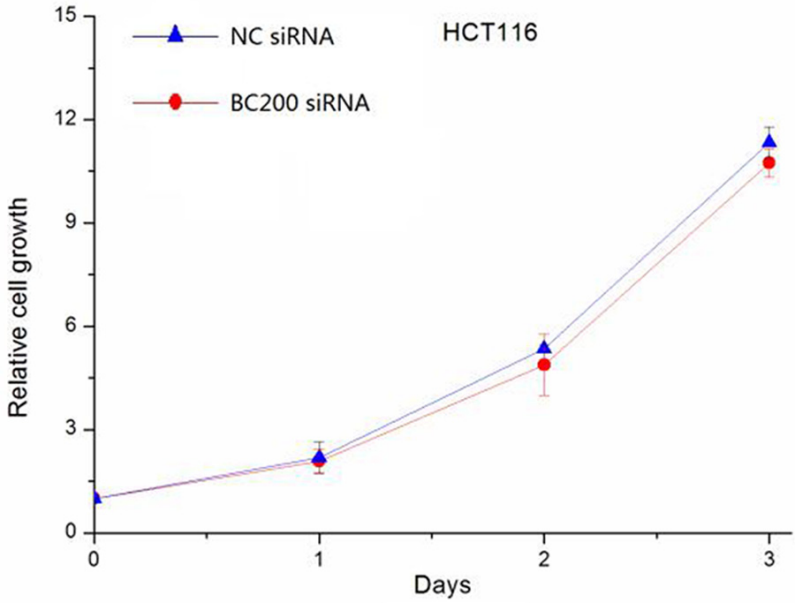

Figure 3. Cell growth was not changed by BC200 RNA siRNA in HCT116 cells.

Carlsbad CA). cDNA synthesis was performed with 2 μg total RNA using the RevertAidTM H Minus First Strand cDNA Synthesis Kit (Takara, Ohtsu, Japan). The primers were obtained from Shenggong (Shanghai, China), and the sequences were as follows: BC200: forward 5'-TGGCTCACGCCTGTAATCC-3; reverse 5'-CCCAGGCAGGTCTCGAACT-3' [21]; EPCAM: forward 5'-AATCGTCAATGCCAGTGTACTT-3', reverse 5'-TCTCATCGCAGTCAGGATCATAA-3'; β-actin: forward 5'-CCACTGGCATCGTGATGGA-3', reverse 5'-CGCTCGGTGAGGATCTTCAT-3'. Quantitative PCR was performed using the SYBR PrimeScript RT-PCR kit (Takara, Ohtsu, Japan) in an Applied Biosystems 7500 Fluorescent Quantitative PCR System (Applied Biosystems, Foster City, CA). The reaction mixtures were incubated at 95°C for 30 s, followed by 40 amplification cycles of 95°C for 5 s and 60°C for 34 s. The comparative CT method was used to quantify relative expression of EPCAM mRNA and BC200 ncRNA. The expression level of a target gene in a patient was calculated as the ratio: target in tumor tissue/target in normal tissue [R (T/N)].

Cell proliferation assay

HCT116 cells were cultured and transfected with BC200 siRNA, or with negative control siRNA as described above. After 48 h, cell proliferation was assessed by MTS assay (Promega) according to the manufacturer's protocol. HCT116 cells (2,000 cells per well) in each group were plated in 96-well plates. Twenty μl of the MTS reagent was added to each well containing 100 μl culture medium. The plate was incubated for 2 h at 37°C in a humidified, 5% CO_2 atmosphere. The plate was then read at 490 nm on a plate reader.

Scratch wound healing assay

Uniform wounds were scraped in HCT116 epithelial monolayers grown on plastic 6-well plates using a pipette tip before transfection. The initial gap length (0 h) and the residual gap

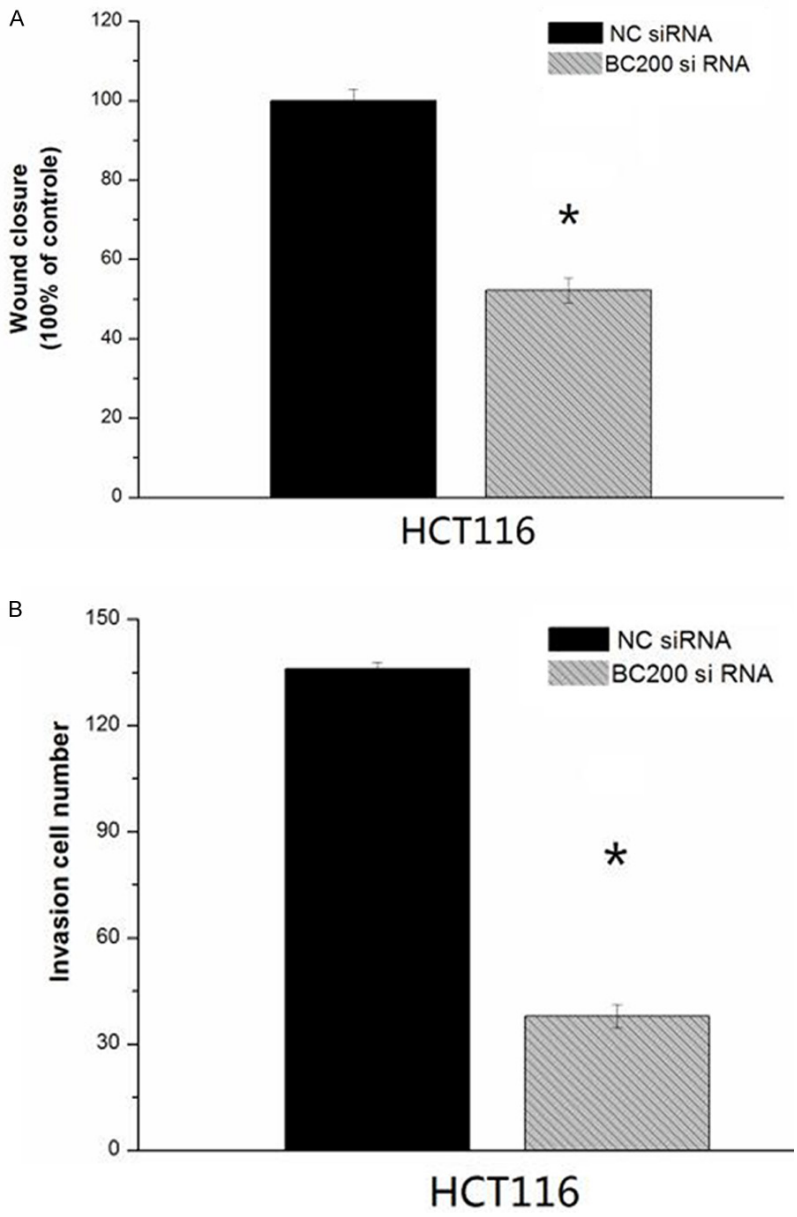

Figure 4. Knockdown of BC200 RNA inhibited the cell migration and invasion.

length 24 h after wounding were calculated from photomicrographs.

Matrigel invasion assays

A cell invasion assay was carried out using modified Boyden Chambers consisting of transwell-precoated Matrigel™ membrane filter inserts with 8 mm pores in 24-well tissue culture plates (BD Biosciences, Bedfold, MA, USA). MEM containing 10% fetal bovine serum in the lower chamber served as the chemoattractant.

Statistical analysis

Differences between groups were analyzed using Student's t test. Correlation between gene expression ratios was studied by using Pearson's correlation. Statistical analyses were performed by using SPSS version 18.0 (SPSS, Chicago, IL). For all statistical analyses, $P < 0.05$ was considered statistically significant.

Results

Expression of BC200 was Co-amplified with EPCAM in CRC tissue samples

The BC200 and EPCAM expression levels were evaluated in the paired specimens in a group of 36 patients with CRC. The expressions of BC200 and EPCAM in CRC tumor tissues were both significantly elevated compared with matched adjacent normal tissue (**Figure 1A**, **1B**). And the results also showed that the expression of BC200 was positively correlated with that of EPCAM in CRC tissue samples ($R = 0.57$, $P < 0.05$). The expression levels of BC200 and EPCAM were both significantly higher in tumor tissues than normal tissues ($P < 0.05$).

Knockdown of BC200 expression by siRNA

After BC200 siRNA was transfected into HCT116 cells, BC200 RNA expression was markedly decreased, which was detected by qPCR. Quantification analysis showed that BC200 RNA expression level was knocked down by about 70% in BC200 siRNA group (**Figure 2**). BC200 levels were measured by qPCR in HCT116 cells transfected with BC200

RNA siRNA or negative control siRNA. Expression of BC200 RNA was significantly reduced in BC200 RNA siRNA groups as compared with negative control groups in HCT116 ($P < 0.05$).

HCT116 cell proliferation was not affected by BC200 RNA knockdown

To explore the effects of BC200 RNA knockdown on the proliferation ability in HCT116 cells, we performed MTS assay in HCT116 cells by suppressing BC200 RNA expression. The results showed that the cell proliferation was not significantly changed in BC200 siRNA group as compared with the cells in the NC group ($P > 0.05$) (**Figure 3**). Cell proliferation fold increase was tested using MTS with 1 day intervals. BC200 RNA siRNA group showed no significant change in the growth rate as compared with negative control group in HCT116 cells ($P > 0.05$).

HCT116 cell migration and invasion were inhibited by BC200 RNA knockdown

To further identify the functional role of BC200 RNA in cell migration and invasion, the scratch wound healing assay and Matrigel™ invasion assay were performed in HCT116 cells in vitro. The wound-healing assay showed remarkable cell migration inhibition in BC200 siRNA group as compared with the cells in the NC group (**Figure 4A**). The Matrigel™ invasion assay also showed significant cell invasion inhibitions in the BC200 siRNA group as compared with NC group in the HCT116 cell line (**Figure 4B**). Scratch wound healing assay and Matrigel™ invasion assay showed that the cell migration and invasion capacity in BC200 siRNA group was remarkably inhibited ($P < 0.05$).

Discussion

Earlier studies have shown that BC200 RNA is commonly overexpressed in multiple malignant tumors. And BC200 has already been identified as a diagnostic marker and a prognostic indicator of invasiveness [15, 16]. However, the expression pattern of BC200 RNA in CRC has not been elucidated yet. The results presented in this paper showed that the expression of BC200 RNA in CRC tumor tissues was significantly higher than that of tumor adjacent normal tissues. It suggests that BC200 RNA may serve as a novel molecular marker in CRC early diagnosis.

BC200 belongs to the non-coding RNA group which has been proved to have extensive regulating activities in different levels of gene expression, and have a crucial biological role in cellular development and metabolism, and in a variety of diseases, including cancer [2-8]. Recently, studies have indicated that a great number of ncRNAs are at adjacent to the protein coding genes [17, 18]. Those ncRNAs located at the upstream of the other adjacent coding gene promoters with a distance of 0.5-2.5 kb, are named promoter upstream transcripts, and often serve as the regulators of the nearby genes [19].

As a translational regulator and a ncRNA, BC200 RNA is just such a promoter upstream transcript of another oncogene-EPCAM. The expression and function of EPCAM is mainly associated with the invasion of malignancies [20, 21] as same as the BC200 RNA in other types of cancers. Therefore, we further explore the expression correlation between BC200 RNA and EPCAM in translational level. By using real-time PCR, we demonstrated that the expression of BC200 RNA was positively correlated with that of EPCAM. It suggests that BC200 RNA may be a regulator of its neighbor gene EPCAM.

Since the expression of BC200 RNA has been clarified, next we explore the possible function of BC200 RNA in CRC. So, whether the function of BC200 RNA is also like that of EPCAM by scratch wound healing assay and Matrigel™ invasion assay, we demonstrated that BC200 RNA may also play an important role in the cell migration and cell invasion. The results showed that the cell migration and invasion capacity in BC200 RNA siRNA group was remarkably inhibited by BC200 siRNA, while the cell proliferation was not evidently changed. It suggests that BC200 RNA is connected with EPCAM not only in their expression levels, but also in their function in CRC cells.

In conclusion, the expression level of BC200 RNA is positively correlated with that of its adjacent oncogene-EPCAM, and the function of BC200 RNA is also associated with invasiveness of CRC cells, just like EPCAM. The overexpression of BC200 RNA may serve as a novel

diagnostic indicator of CRC, and may be utilized as a potential therapeutic target of the invasive tumors in future cancer clinic.

Disclosure of conflict of interest

None.

Address correspondence to: Peng Li, Department of Oncology Surgery, The Chinese PLA General Hospital, Beijing 572013, China; Department of General Surgery, Hainan Branch of the Chinese PLA General Hospital, Sanya, Hainan, China. E-mail: leepeng301@163.com; Xiaohui Du, Department of General Surgery, Hainan Branch of The Chinese PLA General Hospital, Sanya, Hainan, China; Department of General Surgery, The Chinese PLA General Hospital, Beijing, China. E-mail: duxiaohuoi@126.com

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