European Review for Medical and Pharmacological Sciences 2019; 23: 9371-9378

Clinical significance of lncRNA BCYRN1 in colorectal cancer and its role in cell metastasis

J.-H. YU, Y. CHEN

Department of Gastrointestinal Surgery, First Hospital of Jilin University, Changchun, Jilin, China

Abstract. – OBJECTIVE: To explore the expression of long non-coding RNA (lncRNA) BCYRN1 and its clinical significance and function in colorectal cancer (CRC).

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to detect the expression of lncRNA BCYRN1 in CRC tissues and cells. The relationship between lncRNA BCYRN1 expression and CRC clinicopathological characteristics was statistically analyzed. Furthermore, the Kaplan-Meier curve was performed in survival analysis. *In vitro* study, transwell and Western blot assays were used to detect the metastasis ability of CRC cells.

RESULTS: LncRNA BCYRN1 expression was markedly up-regulated in CRC; high expression of lncRNA BCYRN1 promoted tumor metastasis in CRC patients and suggested a poor prognosis. In vitro study, down-regulation of lncRNA BCYRN1 expression by si-BCYRN1 could significantly inhibit invasion and migration of CRC cells.

CONCLUSIONS: LncRNA BCYRN1 was a novel factor involved in CRC progression, and constituted a potential biomarker and therapeutic target for the patients.

Key Words:
Colorectal cancer (CRC), LncRNA BCYRN1, Invasion, Migration, Epithelial-mesenchymal transition (EMT).

Introduction

As fatal cancer worldwide, colorectal cancer (CRC) is one of the most common malignant tumors of the digestive tract. In recent years, the morbidity of CRC has increased and patients have become younger[1]. According to research reports[2], more than 1.2 million new cases of CRC are diagnosed every year, with more than 600 thousand deaths. Although the morbidity of CRC had decreased with the continuous development in the diagnosis and treatment of CRC in recent years, the clinical efficacy for metastatic CRC patients was still low[3]. Moreover, the specific pathogenesis of CRC remains unclear. Therefore, the exploration into biological and epigenetic changes of CRC, especially the detailed molecular mechanism of distant metastasis and discovery of new biomarker and the therapeutic target was of great significance for improving the prognosis of CRC patients[4].

Most of the human genome is transcribed into ribonucleic acids (RNAs), but only 2% of RNAs can encode proteins[5,6]. In transcriptome, RNA molecules that are not translated into proteins are called non-coding RNAs (ncRNAs), while long non-coding RNAs (lncRNAs) are a class of transcribed RNA sequences with a length of more than 200 nucleotides that do not encode proteins. LncRNAs, considered from "the biological waste and noise" to "important regulators during the biological process", had been increasingly recognized as an important part of the genetic program that controls cell differentiation and function. The core of its functions is to be a holder or a bait to collect or isolate effector proteins from its deoxyribonucleic acid (DNA), RNA or protein targets. The effectors regulated by lncRNAs include transcription regulators, chromatin organization, RNA processing and translation, which enable lncRNAs to affect gene expression from multiple levels[7]. In addition, a lot of research evidence showed that lncRNAs were becoming indispensable participators in the occurrence and development of different human tumors[8-10]. Therefore, it was urgent to identify lncRNAs related to the occurrence and development of CRC and to explore their function in CRC.

LncRNA BCYRN1 is a specific lncRNA in brain, neuronal and pluripotent stem cells and is correlated with the release of synaptic neurotransmitters, which is not expressed in other parts under normal conditions[11-13]. However, such neural specificity was broken in tumors[14]. Some research showed that it could be detected in breast cancer and non-small cell lung cancer and its expression was abnormal-

Corresponding Author: Jinhai Yu, MD; e-mail: c_y@jlu.cn

ly increased compared with that in para-carcinoma tissues. Furthermore, it was associated with tumor infiltration, distant metastasis, prognosis and other characteristics[15,16]. However, due to limitations of the present study, whether it was abnormally expressed in CRC and whether it played a role in the disease still need further confirmation.

Patients and Methods

Tissue Specimens Collected

This study was approved by the Ethics Committee, and informed consent was obtained from patients or their family members. Fresh cancerous tissues pathologically diagnosed as CRC in the First Hospital of Jilin University from March 2017 to October 2018 and paired adjacent normal intestinal tissues were collected. Patients did not receive any chemotherapy, radiotherapy and immunotherapy before surgery. The fresh specimens resected by surgery were rapidly placed in cryogenic vials, marked with sample information, cryopreserved in liquid nitrogen and then transferred into a nitrogen canister until RNA extraction in tissues. The clinical data of all patients were complete.

Cell Culture and Transfection

The normal human intestinal epithelial cells (HIECs) and human CRC cell line (SW620) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in the Roswell Park Memorial Institute-1640 (RPMI-1640, Hyclone, South Logan, UT, USA) medium containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) in a sterile incubator under 5% CO_2, 37°C and saturated humidity. The medium was replaced regularly for passage according to the growth of different cells. Then cells were transferred into a 6-well plate (Corning, Lowell, MA, USA) and transfected with si-BCYRN1 (100 pmol) and si-NC (negative control) using Lipofectamine™ 2000 and OPTI-MEMI (Invitrogen, Carlsbad, CA, USA) when 60% of them were fused. After 48 h, the total RNA was extracted for later use.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Analysis

Total RNAs in cells and tissues were extracted using the TRIzol (Invitrogen, Carlsbad, CA, USA) method and reversely transcribed into complementary deoxyribonucleic acid (cDNA) using the PrimeScript™ reverse transcription kit. QRT-PCR was carried out in accordance with the instructions of the SYBR® Premix Ex Taq™ kit (Invitrogen, Carlsbad, CA, USA). ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA) was adopted for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and data collection, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference. (GAPDH: 5'-CAAGGTCATCCATGACAACTTTG-3': 5'-GTCCACCACCCTGTTGCTGTAG-3'; BCYRN1: 5'-CTCAGGGAGGCTAAGAGGCG3', 5'TTTCCTTTTTCTGGAGAACGGG-3').

Western Blot Analysis

The protein extracted from cells using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) and quantified by bicinchoninic acid (BCA) protein quantitative detection kit (Pierce, Waltham, MA, USA), followed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for the protein. The protein was electrically transferred onto a polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland), sealed with 5% skim milk powder at room temperature for 1 h and washed with Tris-Buffered Saline and Tween (TBST; Sigma-Aldrich, St. Louis, MO, USA) for 10 min. Then hybrid membrane was then cut and incubated with E-cadherin and N-cadherin antibody (1:1000, Thermo Fisher Scientific, Waltham, MA, USA) and GAPDH antibody (1:1000, Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight, and it was incubated again with horseradish peroxidase (HRP)-labeled secondary antibody (1:2000). Finally, the protein band was detected using the enhanced chemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA) reagent, photographed using Alpha gel imaging and enhanced chemiluminescence system (ECL) and analyzed using ImageJ analysis software (Media Cybernetics, Silver Springs, MD, USA).

Cell Invasion and Migration

The cells were routinely cultured until the logarithmic growth phase and digested with trypsin. The suspension liquid was washed and added into the upper transwell chamber (about 5×10^4 cells per well) and 200 μL of serum-free basal culture medium was added. Meanwhile, 700 μL of culture medium containing 10% FBS was added into the lower chamber as the chemical inducer. The cells

were cultured routinely in an incubator for 24 h. Then, the cells on the top surface of the filter were wiped off. The filter of the transwell chamber was cut off, fixed with 4% formaldehyde solution (Beyotime, Shanghai, China) and stained with the Giemsa stain. Finally, five high-power fields (200×) were randomly selected to count the number of cells, and the mean value was taken.

The basic steps of the invasion assay were the same as those of the migration assay, except that the invasion chamber should be prepared in advance in the invasion assay. The specific procedures are as follows: Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) diluted with serum-free basal culture medium at 1:3 was overlaid on the bottom of a chamber, and the chamber was placed in the incubator at 37°C. When the gel was solidified, the invasion chamber would be completed. The cells were spread, cultured, fixed and stained in the same way as those of the migration assay.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 13.0 (SPSS, Chicago, IL, USA) software was used for data analysis. The measurement data were analyzed *via* *t*-test. Comparison between groups was made using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). Chi-square test was performed for the association between lncRNA BCYRN1 and clinicopathological parameters of patients, and the influence of lncRNA BCYRN1 on the survival time of patients was evaluated using the Kaplan-Meier method. $p<0.05$ was considered statistically significant.

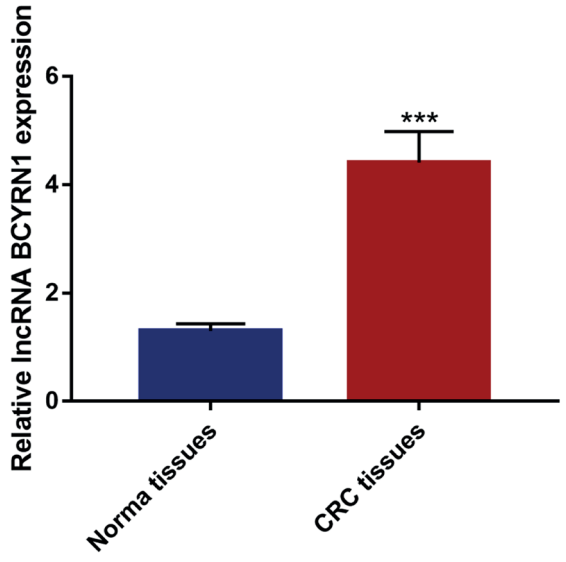

Figure 1. The expression of lncRNA BCYRN1 were measured in CRC and para-cancer tissues by qRT-PCR (***$p<0.001$).

Results

LncRNA BCYRN1 was Highly Expressed in CRC and Promoted CRC Metastasis.

In this study, qRT-PCR was performed to detect the expression level of lncRNA BCYRN1 in 150 pairs of CRC cancer tissues and adjacent normal tissues from patients undergoing surgery in our hospital. The results showed that the expression level of lncRNA BCYRN1 in CRC tissues was significantly higher than that in para-cancer tissues (Figure 1). The mean expression level of lncRNA BCYRN1 in tissues was (2.970 ± 0.385). According to the mean expression level of lncRNA BCYRN1, the patients were divided into the lncRNA BCYRN1 high-expression group (n=79) and low-expression group (n=71). The association between lncRNA BCYRN1 expression and clinicopathological features of patients was analyzed (Table I). The expression level of lncRNA BCYRN1 was associated with regional lymph node (N-stage), distant metastasis (M-stage) and TNM stage of CRC ($p < 0.05$). However, we did not find an absolute association between lncRNA BCYRN1 level and gender, age, tumor location, tumor differentiation, primary tumor (T-stage) and tumor histological type of CRC patients ($p > 0.05$).

Effect of LncRNA BCYRN1 on the Prognosis of Patients with CRC

We performed a 60-month prognostic follow-up of these patients and analyzed their prognosis using the Kaplan-Meier method. Fortunately, no patient was lost to follow-up. The results were shown in Figure 2. Patients with high expression of lncRNA BCYRN1 had a poor prognosis, and their survival curve was significantly weaker than patients with low expression of lncRNA BCYRN1.

Downregulation of LncRNA-BCYRN1 Limited the Invasion and Migration of CRC Cells

To further explore the role of lncRNA BCYRN1 in CRC metastasis, we selected the CRC cell line SW620 for *in vitro* study. First, we examined the differential expression of lncRNA BCYRN1 in SW620 cells and HIECs cells. The results confirmed that lncRNA BCYRN1 also showed a high

Table I. lncRNA BCYRN1 expression and clinical features of colorectal cancer patients.

Features	No.	lncRNA BCYRN1 high	lncRNA BCYRN1 low	p
No.	150	79	71	
Gender				0.868
Male	77	40	37	
Female	73	39	34	
Age (years)				0.813
< 55	93	49	44	
≥ 55	57	30	27	
Location				0.902
Rectum	49	25	24	
Colon	101	54	47	
Tumor differentiation				0.730
Well and moderate	95	50	45	
Poor	55	29	26	
T stage				0.298
T1+T2	89	42	47	
T3+T4	61	37	24	
N stage				0.000
N0	66	19	47	
N1	52	31	21	
N2	32	29	3	
M stage				0.000
M0	104	39	65	
M1	46	40	6	
TNM stage				0.002
I+II	92	37	55	
III+IV	58	42	16	
Tumor Histological				0.794
Adenocarcinoma	111	60	51	
Mucinous adenocarcinoma	39	19	20	

expression level in SW620 cells (Figure 3A). Next, we constructed a low expression group in SW620 cells using si-BCYRN1 (Figure 3B).

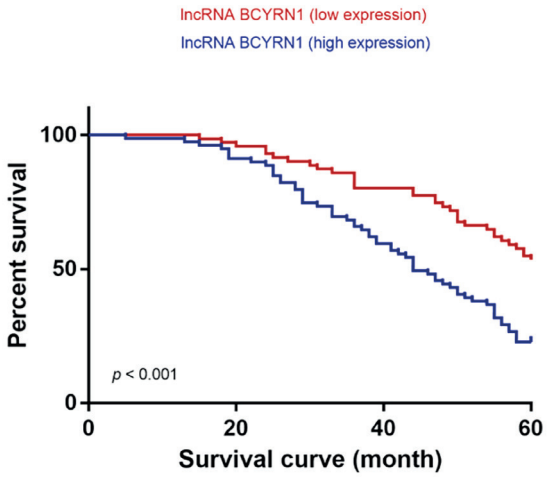

Figure 2. The relationship of lncRNA BCYRN1 expression with overall survival of CRC patients.

Invasion and migration ability was the basic of tumor cells to metastasize[17]; in transwell assay, si-BCYRN1 could significantly reduce the transferability of SW620 cells, and the number of migration and invasion cells in the si-BCYRN1 group was remarkably decreased (Figure 4).

Epithelial-mesenchymal transition (EMT) is another important indicator reflecting the ability of cell metastasis[18]. The EMT associated protein was detected by Western blot experiments. Consistent with what we expected, the results manifested that the expression of the epithelial marker E-cadherin was notably increased, while the expression of the mesenchymal marker N-cadherin was markedly decreased after treatment by si-BCYRN1 (Figure 5).

Discussion

Over the past few decades, much progress has been made in the research about the occurrence

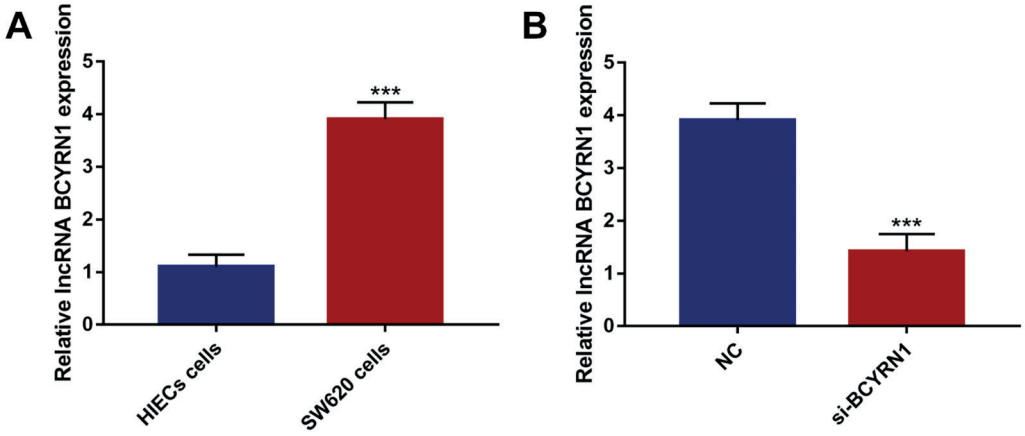

Figure 3. The expression of lncRNA BCYRN1 were measured by qRT-PCR. **A,** Relative expression of lncRNA BCYRN1 in different cells (***p<0.001). **B,** LncRNA BCYRN1 was efficiently knocked down by treatment of si-BCYRN1 (***p<0.001).

and development mechanism of CRC, but there were only a few screening methods for CRC that were hard to be carried out extensively. Therefore, many patients had lymph node metastasis or distant metastasis, evidently affecting the prognosis of patients. Hence, further revealing the molecular mechanism of CRC occurrence and development and exploring the early diagnosis index and therapeutic target was of great significance for improving the prognosis of patients[19].

LncRNA is a newly discovered non-coding RNA that attracts extensive attention since its discovery in recent years, whose function was being continuously revealed. LncRNA could influence the biological properties of tumors, being involved in various processes including chromosome remodeling, transcriptional regulation and RNA degradation. Besides, its sensitivity in the diagnosis of disease was markedly higher than that of DNA, protein-coding RNA and protein

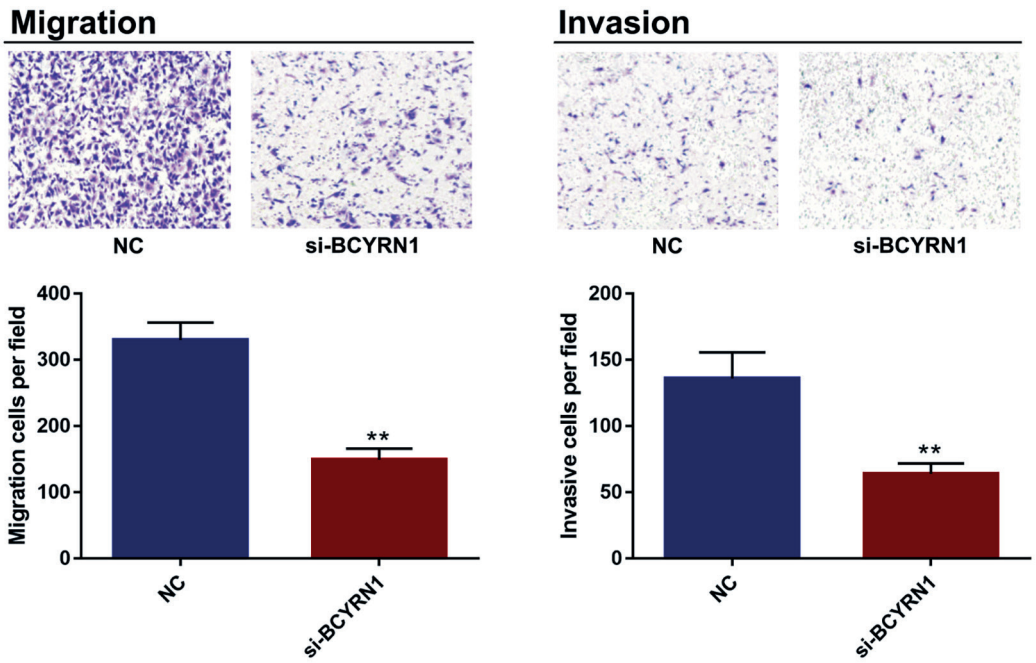

Figure 4. A, Cell invasion and migration was performed using transwell assay and detected by microscope (200×; **p<0.01). **B,** EMT associated protein expression detected by Western blot assay (**p<0.05).

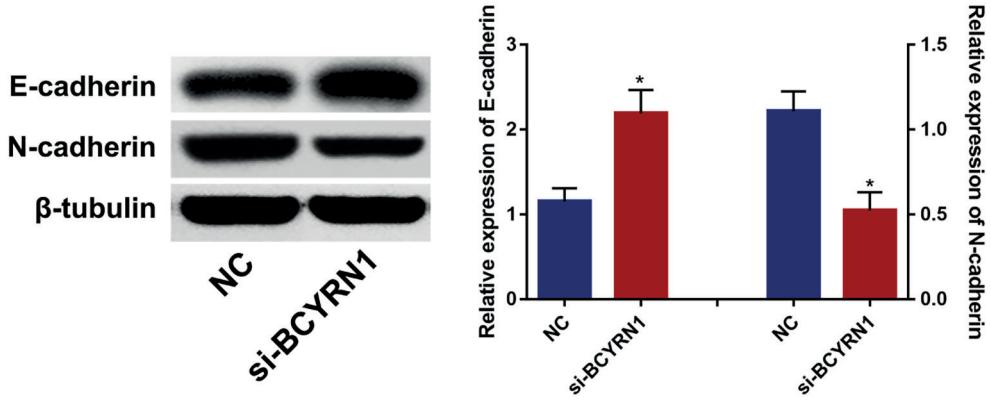

Figure 5. The EMT associated protein was detected by Western blot experiments. si-BCYRN1 notably increased E-cadherin and remarkedly decreased the mesenchymal marker N-cadherin (*p<0.05).

markers due to its tissue specificity[20]. Many studies have reported the role of lncRNA in CRC. For example, the roles of lncRNA H19[21], MALAT1[22], CCAT1[23], CCAT2[24], and HOTAIR[25] in promoting the occurrence and development of CRC had been widely recognized. Therefore, further exploration into the functions of lncRNA in CRC not only had great significance for revealing the occurrence and development mechanism of CRC, but might also provide evidence for searching new diagnosis indexes and therapeutic targets of CRC[20,26,27].

For the first time, our team emphasized the role of lncRNA BCYRN1 in CRC. LncRNA BCYRN1 was up-regulated in CRC and was closely related to the regional lymph node, distant metastasis and TNM stage. It was suggested that lncRNA BCYRN1 was a tumor-promoting gene in CRC, which could promote metastasis of CRC. At the same time, the analysis of our experimental results showed that high expression of lncRNA BCYRN1 in CRC indicated poor prognosis.

One of the key steps in the metastasis cascade of tumors cells is to obtain the invasive ability, including breaking the cell-cell connection, degrading cellular matrix and activating the pathway that controls dynamics of cancer cytoskeleton. EMT is a critical factor in the biological process, in which epithelial cells lose the polarity and are transformed into the mesenchymal phenotype, leading to metastasis of cancer cells[28,29]. EMT could enhance the reaction of cancer cells to environmental triggers, strengthen the invasive ability and benefit the growth and survival of cells. Lots of literature supports that lncRNAs promote or inhibit the occurrence and development of tumors by regulating the EMT signal pathway[30-32].

To further explore the role of lncRNA BCYRN1 in CRC, we interfered with the expression of lncRNA BCYRN1 by si-BCYRN1 in CRC cells *in vitro* to detect its effect on cell metastasis. It was gratifying that reducing the expression of lncRNA BCYRN1 in CRC cells could significantly inhibit the invasion and migration of CRC cells. Furthermore, the progression of EMT in CRC cells was inhibited after lncRNA BCYRN1 expression was reduced.

Our results showed that lncRNA BCYRN1 could promote the metastasis of CRC, and lncRNA BCYRN1 might become a new diagnostic index and therapeutic target for CRC.

Conclusions

Above data suggests that lncRNA CCAT2 is a new biomarker in the carcinogenesis of RCC and CCAT2/miR-320a axis can be served as a candidate target for RCC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Tan HY, Zheng YB, Liu J. Serum miR-199a as a potential diagnostic biomarker for detection of

colorectal cancer. Eur Rev Med Pharmacol Sci 2018; 22: 8657-8663.

2) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.

3) Lin C, Yang L. Long noncoding RNA in cancer: wiring signaling circuitry. Trends Cell Biol 2018; 28: 287-301.

4) Ye C, Shen Z, Wang B, Li Y, Li T, Yang Y, Jiang K, Ye Y, Wang S. A novel long non-coding RNA lncGNAT1-1 is low expressed in colorectal cancer and acts as a tumor suppressor through regulating RKIP-NF-kappaB-Snail circuit. J Exp Clin Cancer Res 2016; 35: 187.

5) Huang G, Wu X, Li S, Xu X, Zhu H, Chen X. The long noncoding RNA CASC2 functions as a competing endogenous RNA by sponging miR-18a in colorectal cancer. Sci Rep 2016; 6: 26524.

6) Venter JC. Genome-sequencing anniversary. The human genome at 10: successes and challenges. Science 2011; 331: 546-547.

7) Alvarez-Dominguez JR, Lodish HF. Emerging mechanisms of long noncoding RNA function during normal and malignant hematopoiesis. Blood 2017; 130: 1965-1975.

8) Li H, Yu B, Li J, Su L, Yan M, Zhu Z, Liu B. Overexpression of lncRNA H19 enhances carcinogenesis and metastasis of gastric cancer. Oncotarget 2014; 5: 2318-2329.

9) Zhu M, Chen Q, Liu X, Sun Q, Zhao X, Deng R, Wang Y, Huang J, Xu M, Yan J, Yu J. lncRNA H19/miR-675 axis represses prostate cancer metastasis by targeting TGFBI. FEBS J 2014; 281: 3766-3775.

10) Loewen G, Jayawickramarajah J, Zhuo Y, Shan B. Functions of lncRNA HOTAIR in lung cancer. J Hematol Oncol 2014; 7: 90.

11) Tiedge H, Chen W, Brosius J. Primary structure, neural-specific expression, and dendritic location of human BC200 RNA. J Neurosci 1993; 13: 2382-2390.

12) Lukiw WJ, Handley P, Wong L, Crapper McLachlan DR. BC200 RNA in normal human neocortex, non-Alzheimer dementia (NAD), and senile dementia of the Alzheimer type (AD). Neurochem Res 1992; 17: 591-597.

13) Mus E, Hof PR, Tiedge H. Dendritic BC200 RNA in aging and in Alzheimer's disease. Proc Natl Acad Sci U S A 2007; 104: 10679-10684.

14) Chen W, Bocker W, Brosius J, Tiedge H. Expression of neural BC200 RNA in human tumours. J Pathol 1997; 183: 345-351.

15) Iacoangeli A, Lin Y, Morley EJ, Muslimov IA, Bianchi R, Reilly J, Weedon J, Diallo R, Bocker W, Tiedge H. BC200 RNA in invasive and preinvasive breast cancer. Carcinogenesis 2004; 25: 2125-2133.

16) Hu T, Lu YR. BCYRN1, a c-MYC-activated long non-coding RNA, regulates cell metastasis of non-small-cell lung cancer. Cancer Cell Int 2015; 15: 36.

17) Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003; 3: 362-374.

18) Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and dissemination precede pancreatic tumor formation. Cell 2012; 148: 349-361.

19) Zhai Z, Yu X, Yang B, Zhang Y, Zhang L, Li X, Sun H. Colorectal cancer heterogeneity and targeted therapy: clinical implications, challenges and solutions for treatment resistance. Semin Cell Dev Biol 2017; 64: 107-115.

20) Slaby O. Non-coding RNAs as biomarkers for colorectal cancer screening and early detection. Adv Exp Med Biol 2016; 937: 153-170.

21) Liang WC, Fu WM, Wong CW, Wang Y, Wang WM, Hu GX, Zhang L, Xiao LJ, Wan DC, Zhang JF, Waye MM. The lncRNA H19 promotes epithelial to mesenchymal transition by functioning as miRNA sponges in colorectal cancer. Oncotarget 2015; 6: 22513-22525.

22) Zheng HT, Shi DB, Wang YW, Li XX, Xu Y, Tripathi P, Gu WL, Cai GX, Cai SJ. High expression of lncRNA MALAT1 suggests a biomarker of poor prognosis in colorectal cancer. Int J Clin Exp Pathol 2014; 7: 3174-3181.

23) Xiang JF, Yin QF, Chen T, Zhang Y, Zhang XO, Wu Z, Zhang S, Wang HB, Ge J, Lu X, Yang L, Chen LL. Human colorectal cancer-specific CCAT1-L lncRNA regulates long-range chromatin interactions at the MYC locus. Cell Res 2014; 24: 513-531.

24) Ling H, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, Nishida N, Gafa R, Song J, Guo Z, Ivan C, Barbarotto E, De Vries I, Zhang X, Ferracin M, Churchman M, van Galen JF, Beverloo BH, Shariati M, Haderk F, Estecio MR, Garcia-Manero G, Patijn GA, Gotley DC, Bhardwaj V, Shureiqi I, Sen S, Multani AS, Welsh J, Yamamoto K, Taniguchi I, Song MA, Gallinger S, Casey G, Thibodeau SN, Le Marchand L, Tiirikainen M, Mani SA, Zhang W, Davuluri RV, Mimori K, Mori M, Sieuwerts AM, Martens JW, Tomlinson I, Negrini M, Berindan-Neagoe I, Foekens JA, Hamilton SR, Lanza G, Kopetz S, Fodde R, Calin GA. CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. Genome Res 2013; 23: 1446-1461.

25) Xue Y, Gu D, Ma G, Zhu L, Hua Q, Chu H, Tong N, Chen J, Zhang Z, Wang M. Genetic variants in lncRNA HOTAIR are associated with risk of colorectal cancer. Mutagenesis 2015; 30: 303-310.

26) Xue Y, Ma G, Gu D, Zhu L, Hua Q, Du M, Chu H, Tong N, Chen J, Zhang Z, Wang M. Genome-wide analysis of long noncoding RNA signature in human colorectal cancer. Gene 2015; 556: 227-234.

27) Han J, Rong LF, Shi CB, Dong XG, Wang J, Wang BL, Wen H, He ZY. Screening of lymph nodes metastasis associated lncRNAs in colorectal cancer patients. World J Gastroenterol 2014; 20: 8139-8150.

28) Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009; 28: 15-33.

29) Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 2010; 29: 4741-4751.

30) Dhamija S, Diederichs S. From junk to master regulators of invasion: lncRNA functions in migration, EMT and metastasis. Int J Cancer 2016; 139: 269-280.

31) Xiao C, Wu CH, Hu HZ. LncRNA UCA1 promotes epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2016; 20: 2819-2824.

32) Zhang W, Yuan W, Song J, Wang S, Gu X. LncRNA CPS1-IT1 suppresses EMT and metastasis of colorectal cancer by inhibiting hypoxia-induced autophagy through inactivation of HIF-1alpha. Biochimie 2018; 144: 21-27.