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LncRNA MIR4435-2HG contributes into colorectal cancer development and predicts poor prognosis

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Abstract. – OBJECTIVE: Some studies have confirmed that long non-coding ribonucleic acids (lncRNAs) played a vital role in the pathophysiology of various diseases, especially in oncogenesis and progression of tumors. Dysexpressed lncRNAs regulate different cellular processes, including proliferation, invasion, and apoptosis. The aim of this study was to explore the clinical significance and function of lncRNA MIR4435-2HG in colorectal cancer.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to detect the expression of lncRNA MIR4435-2HG. The Kaplan-Meier method was used to evaluate the overall survival and disease-free survival of patients with colorectal cancer. The cell proliferation was measured by Methyl thiazolyl tetrazolium (MTT) assay. The cell apoptotic rate was measured *via* flow cytometry method.

RESULTS: LncRNA MIR4435-2HG is a novel cancer-related lncRNA that was recently found to exhibit high expression in colorectal cancer. The dysregulation of lncRNA miR4435-2HG was significantly related to the tumor size ($p<0.001$), lymph node metastasis ($p<0.001$), and tumor node metastasis (TNM) staging ($p=0.022$). The patients with higher expression of lncRNA miR4435-2HG showed worse prognosis than those with low expression of lncRNA miR4435-2HG group. Besides, the downregulated lncRNA miR4435-2HG expression could repress cell proliferation and enhance cell apoptosis.

CONCLUSIONS: LncRNA MIR4435-2HG functions as an oncogene that promotes colorectal cancer progression, and likely represents a biomarker or therapeutic target of colorectal cancer.

Key Words:
LncRNA MIR4435-2HG, Colorectal cancer, Proliferation, Apoptosis.

Introduction

Colorectal cancer is the third most common malignant tumor in the world. More than 693,900 patients die for colorectal cancer each year[1]. The increasing incidence rate of colorectal cancer ranks the fourth of malignant tumors in China and seriously threatens the public's life and health[2]. With the advancement of genomics, it has been found that the development of colorectal cancer is closely related to the changes in proto-oncogenes and tumor suppressor genes[3]. It is reported that about 98% of the human transcripts have no protein-coding ability and were named non-coding RNAs. Among them, the transcripts greater than 200 nt in length are termed long non-coding RNAs (ncRNA)[4]. Scholars[5,6] have shown that lncRNAs functioned epigenetically, transcriptionally, or post-transcriptionally by regulating the expression of the target genes, and thus participating in the development of various diseases, such as diabetes mellitus and tumors. The earliest discovered human lncRNA is H19, which is abnormally expressed in various types of tumors and functions as an oncogene or tumor suppressor gene[7-9]. HOX transcript antisense RNA (HOTAIR) has also been shown to be up-regulated in many types of tumors[10-12]. The high expression of metastasis-associated lung adenocarcinoma transcript 1, MALAT 1, not only promoted the metastasis of lung cancer but was also associated with bladder cancer and colorectal cancer[13-15]. Although accumulated evidence has indicated that lncRNAs might play an important role in tumorigenesis, the regulation of lncRNAs expression in colorectal cancer, and its involvement in tumor formation remain to be elucidated.

Qian et al[16] found that elevated lncRNA miR4435-2HG was significantly correlated with overall survival by evaluating the overall survival of 379 GC patients from The Cancer Genome Atlas (TCGA) database. St Laurent et al[17] also demonstrated that lncRNA miR4435-2HG was highly expressed in lung cancer tissues and cor-

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related with histological grades and lymph node metastasis and promoted lung cancer progression by activating β-catenin signaling. Here, we detected the expression of lncRNA miR4435-2HG in colorectal cancer, analyzed the relationship between MIR4435-2HG expression and clinicopathological factors, and thus explored the potential function and prognosis of lncRNA MIR4435-2HG in colorectal cancer.

Materials and Methods

Collection of Tumor Specimens

The colorectal cancer tissues and adjacent normal tissues from patients in our hospital were collected. After being frozen in liquid nitrogen, all samples were stored at -80°C for further study. Detailed clinical information is summarized in Table I. All patients signed the informed consent, and the collection of specimens was reviewed and approved by our Hospital Ethics Committee.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from the tissues and cells using the TRIzol kit (Invitrogen, Carlsbad, CA, USA), respectively, followed by the measurement of RNA concentration using an ultraviolet spectrophotometer (Hitachi, Tokyo, Japan). The complementary Deoxyribose Nucleic Acid (cDNA) was synthesized according to the instructions of the PrimeScript™ RT MasterMix kit (Invitrogen, Carlsbad, CA, USA). QRT-PCR reaction conditions were as follows: 94°C for 30 s, 55°C for 30 s and72°C for 90 s, for a total of 40 cycles. The relative expression level of the target gene was expressed by the $2^{-\Delta\Delta Ct}$ method. The experiment was repeated for 3 times. Primer sequences used in this study were as follows: lncRNA MIR4435-2HG, F: 5'-CGGAGCCACGACCACTTCCTC-3', R: 5'-GGCTCGGATATGTCAGACGCA-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Cell Culture and Transfection

The cancer cell lines were cultured with Roswell Park Memorial Institute-1640 (RPMI-1640) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Grand Island, NY, USA) in a 5% CO_2 and at 37°C humidified incubator.

Both of siRNA-lncRNA miR4435-2HG and siRNA-NC were synthesized and purified by Shanghai GenePharma Co., Ltd. (Shanghai, China). The cells were seeded into a 6-well plate (Corning, Corning, NY, USA) at a density of

Table I. The relationship between the expression level of lncRNA miR4435-2HG and the clinicopathological features of colorectal cancer patients.

Parameters	Total (n)	miR4435-2HG Low (n)	miR4435-2HG High (n)	*p*-value
Age (years)				
<60	48	22	26	
≥60	54	25	29	0.963
Gender				
male	53	26	27	
female	49	21	28	0.530
Tumor size (cm)				
<4	47	32	15	
≥4	55	15	40	<0.001*
Tumor differentiation				
G1	37	20	17	
G2/G3	65	27	38	0.223
TNM stage				
I-II	57	32	25	
III-IV	45	15	30	0.022*
Lymph node metastasis				
no	50	33	17	
yes	52	14	38	<0.001*

*$p<0.05$

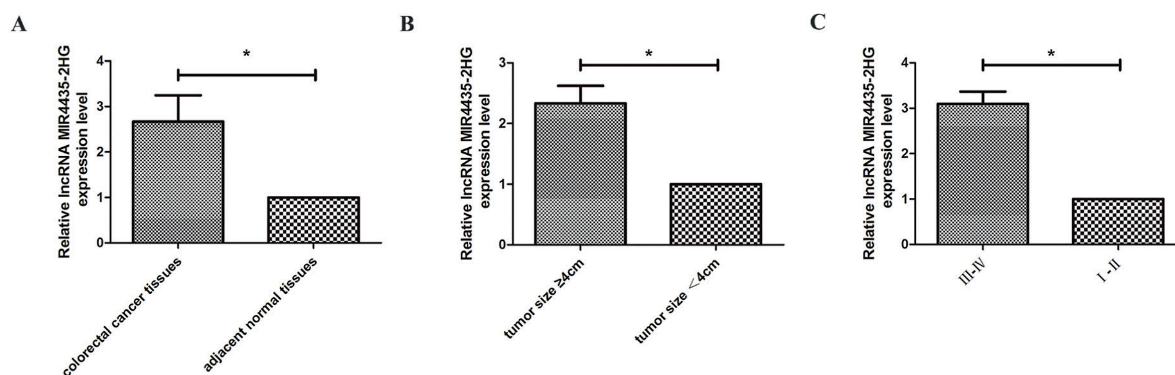

Figure 1. Detection of lncRNA miR4435-2HG expression level in colorectal cancer tissues. **A,** The expression level of lncRNA miR4435-2HG in colorectal cancer and adjacent normal tissues was detected by qRT-PCR. **B,** The expression level of lncRNA miR4435-2HG in tissues from tumor size ≥ 4 cm group and tumor size < 4 cm group was detected by qRT-PCR. **C,** The expression level of lncRNA miR4435-2HG in III-IV group and I-II group was detected by qRT-PCR. *$p<0.05$.

5×10^5 cells/well. After 48 h, siRNA-lncRNA miR4435-2HG and siRNA-NC were transfected into cell lines with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction.

Methyl Thiazolyl Tetrazolium (MTT) Assays

The cells were seeded and incubated in a 96-well plate at a density of 1×10^3 cells/well. Totally 20 μL MTT (Beyotime, Shanghai, China) reagent was added into each well, the cells were incubated for other 4 h. Then, 150 μL dimethyl sulfoxide (DMSO) was added to each well. Finally, the absorbance wavelength was measured.

Cell Apoptosis

Cell apoptosis was measured using an Annexin V-FITC (fluorescein isothiocyanate) Kit (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Totally, 5×10^5 cells were re-suspended in the binding buffer (200 μL), which was then incubated with FITC Annexin V (5 μL) and propidium iodide solution (1 μL). The apoptosis rate of cells was measured by FACS Calibur Flow Cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Statistical Analysis

After the collection and organization of clinical pathological information and prognostic data, the effect of lncRNA MIR4435-2HG expression level on clinicopathological factors was analyzed. The survival curve was drawn by the Kaplan-Meier method. The survival rate between lncRNA miR4435-2HG high expression group and low expression group was also compared. The Cox proportional hazard model was used for multivariate analysis to evaluate the role of lncRNA MIR4435-2HG on the prognosis of colorectal cancer. All the above relevant data were statistically analyzed using statistical software Statistical Product and Service Solutions (SPSS) 21.0 (IBM Corp., Armonk, NY, USA) statistical software.

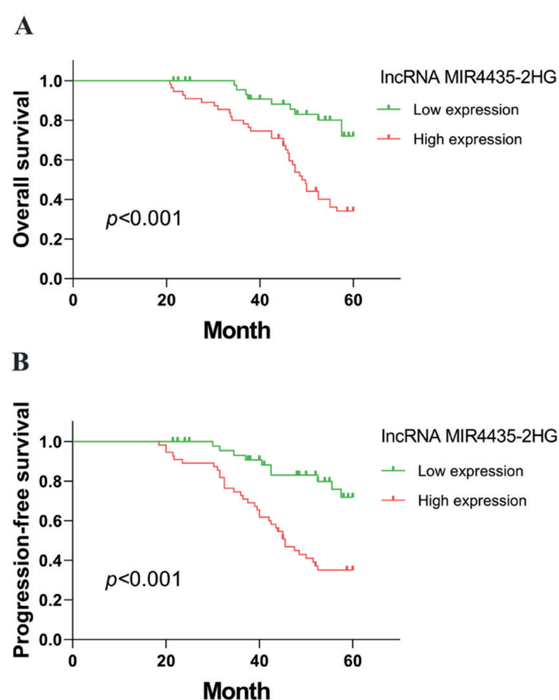

Figure 2. Relationship between the expression level of lncRNA miR4435-2HG and prognosis. To further analyze the relationship between the expression level of lncRNA miR4435-2HG and the prognosis of patients with colorectal cancer, the Kaplan-Meier survival analysis, and the log-rank analysis were performed. **(A)** Overall Survival, OS, **(B)** Disease-Free Survival, DFS.

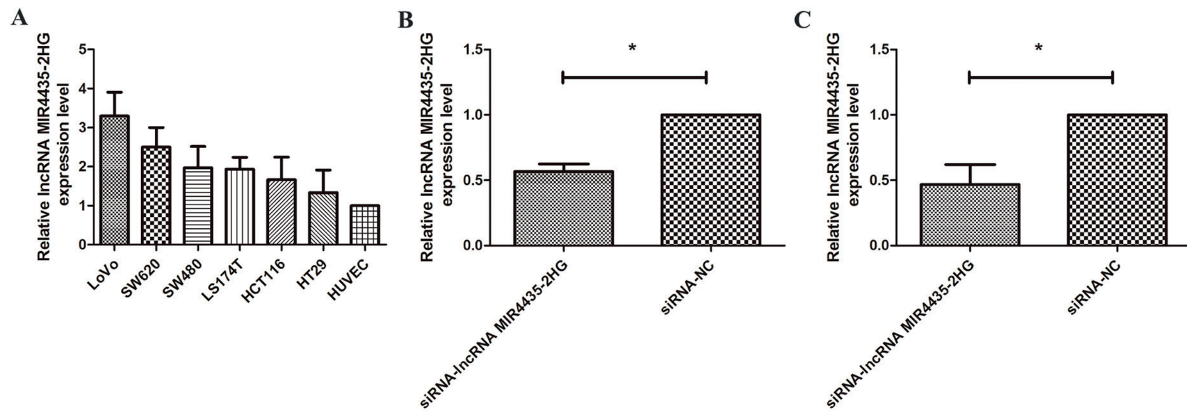

Figure 3. Downregulation of lncRNA miR4435-2HG in colorectal cancer cells by transfecting with siRNA. **A,** The expression level of lncRNA miR4435-2HG was measured by qRT-qPCR. **B,** (LoVo) and C (SW620), the cells were transfected with siRNA-lncRNA miR4435-2HG. The transfection effects were confirmed qRT-qPCR. *$p<0.05$.

Results

Detection of LncRNA MIR4435-2HG Expression Level in Colorectal Cancer Tissues

The expression level of lncRNA MIR4435-2HG in colorectal cancer and adjacent normal tissues was detected by qRT-PCR. The results showed that the expression of lncRNA miR4435-2HG was significantly higher in tumor tissues than that in the adjacent normal tissues (Figure 1A). In addition, lncRNA MIR4435-2HG showed elevated expression in patients with tumor size ≥ 4 cm than those with tumor size < 4 cm (Figure 1B). Similarly, lncRNA MIR4435-2HG expression also increased in tumor tissues of III-IV patients than that of I-II patients (Figure 1C). Furthermore, the relationship between the expression level of lncRNA MIR4435-2HG and the clinicopathological features of patients with colorectal cancer was also analyzed. As shown in Table I, significant differences were observed between the lncRNA MIR4435-2HG expression level and the tumor size ($p<0.001$), lymph node metastasis ($p<0.001$), tumor node metastasis (TNM) staging ($p=0.022$), while there was no remarkable difference between the lncRNA MIR4435-2HG expression level and the patient's gender, age, tumor differentiation ($p>0.05$).

Relationship Between Expression Level of LncRNA MIR4435-2HG and Prognosis

To further analyze the relationship between the expression level of lncRNA MIR4435-2HG and the prognosis of patients with colorectal cancer, the Kaplan-Meier survival analysis, and the log-rank analysis were performed. The results showed that the patients in lncRNA miR4435-2HG higher expression group showed a worse prognosis compared to the lncRNA miR4435-2HG low expression group (Figure 2A). The disease-free survival (DFS) of lncRNA miR4435-2HG high expression group was also significantly lower than that of lncRNA miR4435-2HG low expression group (Figure 2B). The regression of Cox risk ratio revealed that tumor size ($p=0.007$, HR=2.756), TNM stage ($p=0.008$, HR=3.203), lymph node metastasis ($p=0.031$, HR=3.333), and lncRNA miR4435-2HG ($p=0.015$, HR=2.462) resulted in an increased risk of death (Table II). These results suggested that the increased expression of lncRNA MIR4435-2HG might be an independent indicator of the prognosis of colorectal cancer patients.

Downregulation of LncRNA MIR4435-2HG in Cell Lines by Transfecting with SiRNA

The expression level of lncRNA MIR4435-2HG in colorectal cancer cells (LoVo, SW620, SW480, LS174T, HCT116, and HT29) and normal human colorectal cells (HUVEC) was measured in this study. The analysis of qRT-qPCR results revealed that lncRNA miR4435-2HG expression was higher in colorectal cancer cells (LoVo, SW620, SW480, LS174T, HCT116, and HT29) than that in the normal human colorectal cells (HUVEC) (Figure 3A). Thus, LoVo and SW620 cell lines were chosen for further exploration in this study. Due to the increased expression of lncRNA miR4435-2HG in colorectal cancer cell lines, siRNA-lncRNA miR4435-2HG was synthe-

sized and transfected into LoVo and SW620 cells. The interference effects were then observed, as shown in Figures 3B and 3C.

Downregulated LncRNA MIR4435-2HG Expression Repressed Cell Proliferation and Enhanced Cell Apoptosis

After lncRNA miR4435-2HG was down-regulated *in vitro*, MTT assay was used to detect the cell proliferation of colorectal cancer cells. The results demonstrated that the proliferation of cells transfected with siRNA-lncRNA miR4435-2HG was markedly downregulated compared with the cells transfected with siRNA-NC (p<0.05, Figures 4A, 4B). In addition, the apoptosis rate was measured using flow cytometry method, and the results revealed that reduced lncRNA miR4435-2HG remarkably increased the apoptosis of colorectal cancer cells (p<0.05, Figures 4C and 4D). All the results indicated that the down-regulated lncRNA miR4435-2HG could repress cell proliferation and enhance cell apoptosis.

Discussion

LncRNAs were used to be considered as the "genomic noise"[18], while subsequent studies have

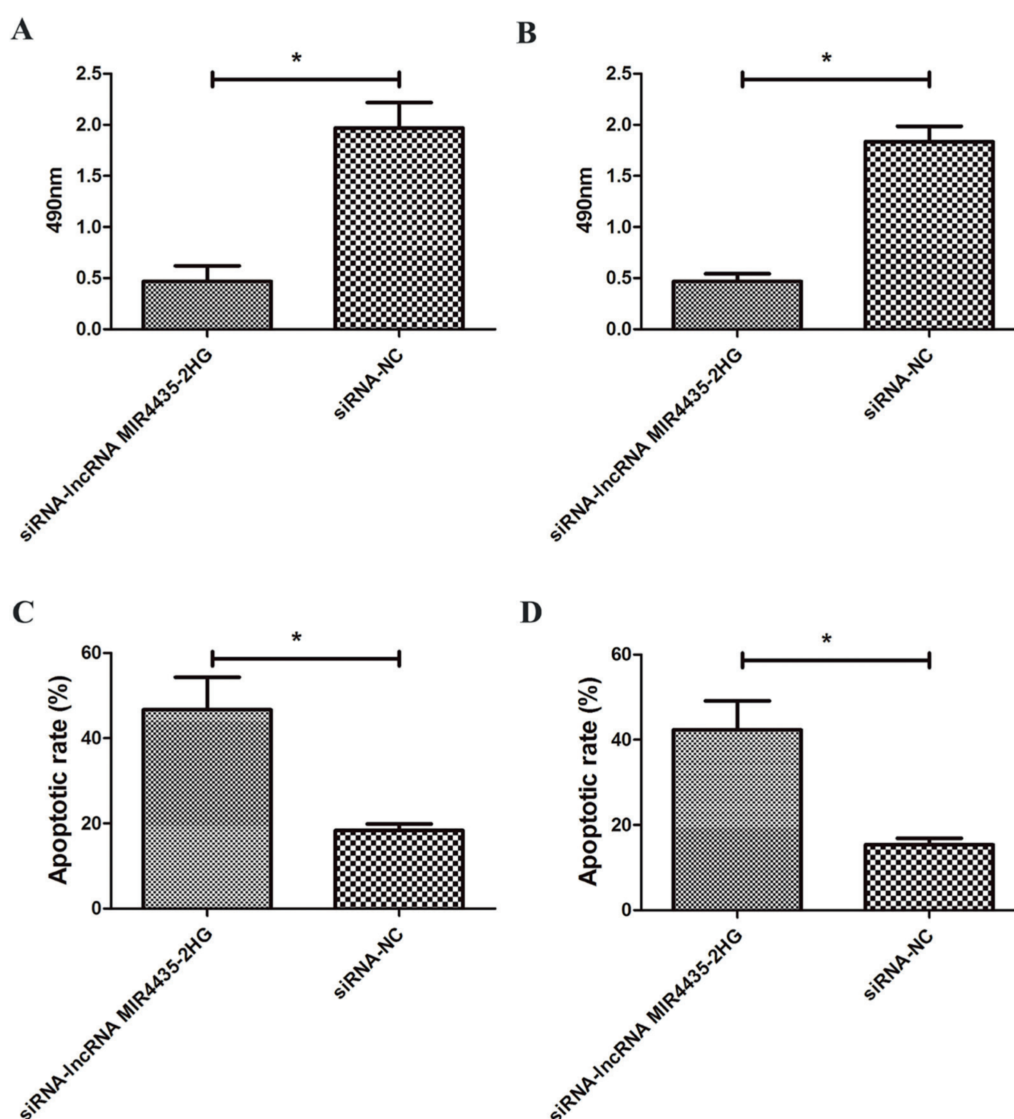

Figure 4. Downregulated lncRNA miR4435-2HG expression could repress cell proliferation and enhance cell apoptosis. **(A)** (LoVo) and **(B)** (SW620), MTT assays were used to detect the cell proliferation. **(C)** (LoVo) and **(D)** (SW620), the cell apoptotic rate was measured *via* using flow cytometry. *p<0.05.

confirmed that lncRNA was involved in the development of various diseases *via* regulating the expression of the target genes through epigenetics, transcriptional, and post-transcriptional regulation[19]. The activation of proto-oncogenes and the inactivation of tumor suppressor genes will ultimately contribute to tumorigenesis[20]. Therefore, studies on lncRNAs associated with tumorigenesis would be a benefit for the discovery of new biomarkers for tumor diagnosis and prognosis.

To analyze the expression of lncRNA miR4435-2HG in colorectal cancer, qRT-PCR was performed, and the results revealed that lncRNA miR4435-2HG was significantly up-regulated in colorectal cancer compared with adjacent normal tissues. LncRNAs were closely related to the clinical features and prognosis of colorectal cancer. LncRNA-GAPLINC was highly expressed in colorectal cancer and was associated with tumor size, T grade, and N grade, and was also identified as an independent risk factor for the prognosis of colorectal cancer[21]. Patients with colorectal cancer with high expression of lncRNA-H19 have a lower degree of tumor differentiation, a later TNM stage, shorter overall survival, and disease-free survival[22]. The clinical pathological analysis found that the expression of lncRNA miR4435-2HG in colorectal cancer tissues was related to the tumor size of colorectal cancer. The survival analysis also revealed that patients with colorectal cancer with low expression of lncRNA miR4435-2HG had longer disease-free survival than those with high expression of lncRNA miR4435-2HG. The above results demonstrated that the higher the malignancy of colorectal cancer, the higher the expression of lncRNA miR4435-2HG, and the worse the prognosis of patients.

LncRNA CRNDE was reported to promote colorectal cancer cell proliferation and chemoresistance *via* miR-181a-5p-mediated regulation of Wnt/β-catenin signaling[23]. Long non-coding RNA UICLM also contributed to the colorectal cancer liver metastasis by acting as a ceRNA for microRNA-215 to regulate ZEB2 expression[24]. Long noncoding RNA XIST expedited the metastasis and modulated the epithelial-mesenchymal transition in colorectal cancer[25]. Long non-coding RNA CCAL regulated the colorectal cancer progression by activating Wnt/β-catenin signaling pathway *via* suppression of the activator protein 2α[26]. The downregulation of LncRNA-ANCR suppressed the invasion and migration of colorectal cancer cells by regulating the EZH2 expression[27]. In this study, the expression of lncRNA miR4435-2HG in cell lines was inhibited by transfecting with siRNA. Through MTT and flow cytometry analysis, we found that the down-regulated lncRNA MIR4435-2HG could repress the cell proliferation and enhance cell apoptosis. Therefore, we confirmed that lncRNA miR4435-2HG played an important role in the development of colorectal cancer.

Conclusions

We detected that the high expression of lncRNA miR4435-2HG in colorectal cancer tissues was related to tumor size, which might have a suggestive effect on the diagnosis and prognosis of colorectal cancer. A subsequent study of the mechanism of lncRNA miR4435-2HG involved in the development of colorectal cancer had a certain value for the improvement of early diagnosis, treatment, and prognosis of colorectal cancer.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Chen W, Zheng R, Zeng H, Zhang S, He J. Annual report on status of cancer in China, 2011. Chin J Cancer Res 2015; 27: 2-12.
3) Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487: 330-337.
4) Costa FF. Non-coding RNAs: lost in translation? Gene 2007; 386: 1-10.
5) Jaé N, Dimmeler S. Long noncoding RNAs in diabetic retinopathy. Circ Res 2015; 116: 1104-1106.
6) Martens-Uzunova ES, Bottcher R, Croce CM, Jenster G, Visakorpi T, Calin GA. Long noncoding RNA in prostate, bladder, and kidney cancer. Eur Urol 2014; 65: 1140-1151.
7) Luo M, Li Z, Wang W, Zeng Y, Liu Z, Qiu J. Long non-coding RNA H19 increases bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin expression. Cancer Lett 2013; 333: 213-221.
8) Zhang L, Yang F, Yuan JH, Yuan SX, Zhou WP, Huo XS, Xu D, Bi HS, Wang F, Sun SH. Epigenetic activation of the miR-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. Carcinogenesis 2013; 34: 577-586.

9) Berteaux N, Aptel N, Cathala G, Genton C, Coll J, Daccache A, Spruyt N, Hondermarck H, Dugimont T, Curgy JJ, Forné T, Adriaenssens E. A novel H19 antisense RNA overexpressed in breast cancer contributes to paternal IGF2 expression. Mol Cell Biol 2008; 28: 6731-6745.

10) Zhang JX, Han L, Bao ZS, Wang YY, Chen LY, Yan W, Yu SZ, Pu PY, Liu N, You YP, Jiang T, Kang CS, Chinese Glioma Cooperative Group. HOTAIR, a cell cycle-associated long noncoding RNA and a strong predictor of survival, is preferentially expressed in classical and mesenchymal glioma. Neuro Oncol 2013; 15: 1595-1603.

11) Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 2011; 71: 6320-6326.

12) Niinuma T, Suzuki H, Nojima M, Nosho K, Yamamoto H, Takamaru H, Yamamoto E, Maruyama R, Nobuoka T, Miyazaki Y, Nishida T, Bamba T, Kanda T, Ajioka Y, Taguchi T, Okahara S, Takahashi H, Nishida Y, Hosokawa M, Hasegawa T, Tokino T, Hirata K, Imai K, Toyota M, Shinomura Y. Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors. Cancer Res 2012; 72: 1126-1136.

13) Gutschner T, Hämmerle M, Eissmann M, Hsu J, Kim Y, Hung G, Revenko A, Arun G, Stentrup M, Gross M, Zörnig M, MacLeod AR, Spector DL, Diederichs S. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res 2013; 73: 1180-1189.

14) Ying L, Chen Q, Wang Y, Zhou Z, Huang Y, Qiu F. Upregulated MALAT-1 contributes to bladder cancer cell migration by inducing epithelial-to-mesenchymal transition. Mol Biosyst 2012; 8: 2289-2294.

15) Xu C, Yang M, Tian J, Wang X, Li Z. MALAT-1: a long non-coding RNA and its important 3' end functional motif in colorectal cancer metastasis. Int J Oncol 2011; 39: 169-175.

16) Ke D, Li H, Zhang Y, An Y, Fu H, Fang X, Zheng X. The combination of circulating long noncoding RNAs AK001058, INHBA-AS1, MIR4435-2HG, and CEBPA-AS1 fragments in plasma serve as diagnostic markers for gastric cancer. Oncotarget 2017; 8: 21516-21525.

17) Qian H, Chen L, Huang J, Wang X, Ma S, Cui F, Luo L, Ling L, Luo K, Zheng G. The lncRNA miR4435-2HG promotes lung cancer progression by activating beta-catenin signalling. J Mol Med (Berl) 2018; 96: 753-764.

18) St Laurent G, Wahlestedt C, Kapranov P. The landscape of long noncoding RNA classification. Trends Genet 2015; 31: 239-251.

19) Zhao Y, Qin ZS, Feng Y, Tang XJ, Zhang T, Yang L. Long non-coding RNA (lncRNA) small nucleolar RNA host gene 1 (SNHG1) promote cell proliferation in colorectal cancer by affecting P53. Eur Rev Med Pharmacol Sci 2018; 22: 976-984.

20) Wang Y, Velho S, Vakiani E, Peng S, Bass AJ, Chu GC, Gierut J, Bugni JM, Der CJ, Philips M, Solit DB, Haigis KM. Mutant N-RAS protects colorectal cancer cells from stress-induced apoptosis and contributes to cancer development and progression. Cancer Discov 2013; 3: 294-307.

21) Yang P, Chen T, Xu Z, Zhu H, Wang J, He Z. Long noncoding RNA GAPLINC promotes invasion in colorectal cancer by targeting SNAI2 through binding with PSF and NONO. Oncotarget 2016; 7: 42183-42194.

22) Han D, Gao X, Wang M, Qiao Y, Xu Y, Yang J, Dong N, He J, Sun Q, Lv G, Xu C, Tao J, Ma N. Long noncoding RNA H19 indicates a poor prognosis of colorectal cancer and promotes tumor growth by recruiting and binding to eIF4A3. Oncotarget 2016; 7: 22159-22173.

23) Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, Liu YL, Cui BB. The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/beta-catenin signaling. Mol Cancer 2017; 16: 9.

24) Chen DL, Lu YX, Zhang JX, Wei XL, Wang F, Zeng ZL, Pan ZZ, Yuan YF, Wang FH, Pelicano H, Chiao PJ, Huang P, Xie D, Li YH, Ju HQ, Xu RH. Long non-coding RNA UICLM promotes colorectal cancer liver metastasis by acting as a ceRNA for microRNA-215 to regulate ZEB2 expression. Theranostics 2017; 7: 4836-4849.

25) Chen DL, Chen LZ, Lu YX, Zhang DS, Zeng ZL, Pan ZZ, Huang P, Wang FH, Li YH, Ju HQ, Xu RH. Long noncoding RNA XIST expedites metastasis and modulates epithelial-mesenchymal transition in colorectal cancer. Cell Death Dis 2017; 8: e3011.

26) Ma Y, Yang Y, Wang F, Moyer MP, Wei Q, Zhang P, Yang Z, Liu W, Zhang H, Chen N, Wang H, Wang H, Qin H. Long non-coding RNA CCAL regulates colorectal cancer progression by activating Wnt/β-catenin signalling pathway via suppression of activator protein 2α. Gut 2016; 65: 1494-1504.

27) Yang ZY, Yang F, Zhang YL, Liu B, Wang M, Hong X, Yu Y, Zhou YH, Zeng H. LncRNA-ANCR down-regulation suppresses invasion and migration of colorectal cancer cells by regulating EZH2 expression. Cancer Biomark 2017; 18: 95-104.