European Review for Medical and Pharmacological Sciences 2018; 22: 1658-1664

Effects of long non-coding RNA URHC on proliferation, apoptosis and invasion of colorectal cancer cells

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Abstract. – OBJECTIVE: To investigate the effect of long non-coding RNA URHC on the proliferation, apoptosis and invasion of colorectal cancer cells.

PATIENTS AND METHODS: The expression of lncRNA-URHC in tissues and cells was tested by Real-time quantitative PCR. The expression of lncRNA-URHC was down-regulated by RNA [illegible] ference (siRNA). The Real-time quantitati[illegible] merase chain reaction (PCR) method was [illegible] to detect the interference efficiency. Cell cou[illegible] kit-8 (CCK-8), flow cytometry, and transwell [illegible] used to detect the effect of lncRNA-URHC on t[illegible] proliferation, apoptosis and inv[illegible] of colorec[illegible] tal cancer cells. The effect o[illegible] RHC on epithelial-mesenchymal tra[illegible]on (E[illegible]related markers was detected by [illegible]tern blo[illegible]

RESULTS: LncRNA-U[illegible] xpres[illegible] nificantly increased i[illegible] co[illegible]ncer cells compared with norm[illegible]ls, an[illegible]xpression of lncRNA-URHC in c[illegible]ectal cance[illegible] was higher than that in th[illegible]al cell. After d[illegible]egulated the expres[illegible]NA-URHC, the proliferation and inva[illegible] of co[illegible]al cancer cells were decreased, while cells ap[illegible]s was promoted. Down-reg[illegible]on of lncRNA-U[illegible] could enhance the exp[illegible]ion of E-cadherin an[illegible] reduce the expression of N-ca[illegible]in, vimentin and snail.

C[illegible]LUSIO[illegible] Down regulation of lncRNA-URH[illegible]it the [illegible]gression of colorectal can[illegible]

Key words:
C[illegible]ectal ca[illegible]ong non-coding RNA-URHC, Cell [illegible]feration, Cell invasion, EMT.

Introduction

[illegible]ectal cancer is regarded as one type of the [illegible]t common malignancies. Although the t[illegible]onal treatment methods, such as surgical [illegible]ction, radioth[illegible]apy chemotherapy, and target-[illegible]therapy tech[illegible]gy are improving, its 5-year [illegible]ival rate is s[illegible]ow[1]. Therefore, it is urgent to i[illegible]ate the [illegible]hanisms of colorectal cancer cell [illegible] and metastasis process, to find new molecular targets, and to improve the clinical [illegible]eatment effects.

[illegible]NAs are noncoding RNAs greater [illegible]an [illegible]0 nt in length and participate in the development of various diseases[2-4]. In recent years, the researchers[5] found that lncRNA in malignant tumors in the abnormal expression, and tumor cell proliferation, invasion, metastasis and other biological processes, are closely related. LncRNA H19 was able to serve as a competing endogenous gene to regulate EMT-related genes, so that break the regulatory association of miRNA and EMT[6]. In the patients with colorectal cancer, upregulation of lncRNA-ATB was involved in metastasis and tumor size, and might be a potential marker of poor prognosis[7]. High expression of lncRNA-BANCR was contributed into the patients with gastric cancer clinical characters[8]. In gastric cancer, abnormal expression of Linc00152 is associated with cell apoptosis, cycle arrest, cell migration, invasion[9].

However, the current expression and function of lncRNA-URHC in colorectal cancer is not clear. This article will focus on lncRNA-URHC in cancer tissues and cells in the expression of the initial exploration of its colorectal cancer cell proliferation, apoptosis and invasion process, for clinical diagnosis and treatment to provide a strong theoretical basis.

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Patients and Methods

Patients

All specimens of cancer tissue and its adjacent matching tissue were collected from our hospital in patients with colorectal cancer after surgery. Then, specimens were quickly placed into the liquid nitrogen, and immediately saved in -80°C ultra-low temperature refrigerator. This study was approved by the Ethics Committee of the First People's Hospital of Wujiang District Suzhou. Signed written informed consents were obtained from all participants before the study.

Materials and Reagents

Colorectal cancer cells and normal cells were purchased from Shanghai Institute of Biochemistry and Cell Biology, Institute of Life Sciences (Shanghai, China). Roswell Park Memorial Institute-1640 (RPMI-1640) medium and fetal bovine serum (FBS) were purchased from Gibco Corporation (Rockville, MD, USA), RNA extraction reagent TRIzol and reverse transcription kit were purchased from TaKaRa Corporation (Dalian, China), lncRNA-URHC in[illegible]on RNA (siRNA lncRNA-URHC) and [illegible] control si-Negative Control (si-NC) we[illegible]btained by Shanghai Gemma Co., Ltd., (Shan[illegible] China). Cell transfection reagent Lipofectam[illegible] 2000 and cell counting kit-8 (CCK-8) reage[illegible] were purchased from Invitro[illegible]bad, CA, USA), matrigel matrix and [illegible]nswe[illegible]hamber were purchased from BD [illegible]anklin [illegible]es, NJ, USA), dimethyl sulfoxi[illegible]stalli[illegible] and phosphate-buffer[illegible]l sa[illegible] were purchased from HyCl[illegible] Compa[illegible]uth Logan, UT, USA).

Methods

Real-t[illegible] Quantitative PCR

Th[illegible]pressi[illegible] lncRNA-URHC and glyceraldh[illegible]3-p[illegible]nate d[illegible]drogenase (GAPDH) in tis[illegible] cells w[illegible] detected by Taqman p[illegible]be me[illegible]. The e[illegible]ssion of lncRNA-URHC [illegible]APDH[illegible]es and cells were detected [illegible]RIzol m[illegible]d. lncRNA-URHC upstream prim[illegible]5'-TGTTTATGTGAGAGGAGAAAGG[illegible]stream primer: 5'-CACTAGAG-[illegible]TGCAAATAAAGTGA-3'. GAPDH up-[illegible]m primer: 5'-ACCCAGAAGACTGTGGAT-C[illegible]downstream primer: 5'-TTCTAGACGG-CAC[illegible]CAGGT-3'.

Cell Transfection Experiments

Cells were cultured in medium (R[illegible] containing 10% FBS and placed i[illegible]5% C[illegible] at 37°C incubator. When the ce[illegible]ere in log-arithmic growth phase, they [illegible]eeded in a six-well plate at a density of about [illegible]ith 5 μL of Lipofectamine 2000 an[illegible]00 pmo[illegible]A ln[illegible] cRNA-URHC mixture. A[illegible]e same time[illegible] Lipofectamine 2000 a[illegible]i-NC w[illegible]re added[illegible] cells in the control g[illegible].

Cell Proliferat[illegible]in[illegible]Expe[illegible]ents

The transfe[illegible] cells w[illegible]eed[illegible]m plates (96-well) an[illegible] μL of 10% [illegible]vine serum (FBS) me[illegible] added to eac[illegible]ell to ensure that the [illegible]ber o[illegible]per well was about 2,000. After incubation for [illegible]48 h and 72 h, 10 μL of C[illegible]eagent were a[illegible] to each well. After i[illegible]ation for 2 h, OD values were measured at [illegible]nm. Each gro[illegible] was repeated three times.

[illegible]w Cytomet[illegible]Assay

[illegible] transfe[illegible]n for 24 h, cells apoptosis was dete[illegible]nnexin V labeling. An annexin V-APC labeled Apoptosis Detection Kit (Abcam, Cambridge, MA, USA) was purchased; next, we [illegible]flow cytometry assay according to the [illegible]otoc[illegible].

Cell Invasion Transwell Experiment

The Matrigel gel was diluted with 10% FBS RPMI-1640 medium at a ratio of 1:5 and 50 μL of diluent were spread evenly in the transwell chamber. Cells in logarithmic growth phase were digested and resuspended in 10% FBS RPMI-1640 medium and added to the transwell chamber. The number of cells in each cell was about 2×10^4 cells. 700 μL of medium containing 10% FBS were cultured in a cell incubator for 24 h. The medium was discarded, washed three times with PBS, fixed with anhydrous methanol for 20 min, stained with crystal violet dye for 30 min, and placed under a microscope randomly selected five view camera count.

Statistical Analysis

SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. All quantitative data were expressed as mean ± standard deviation. Comparison between groups was done using One-way ANOVA test followed by least significant difference (LDS). $p < 0.05$ as the difference was statistically significant.

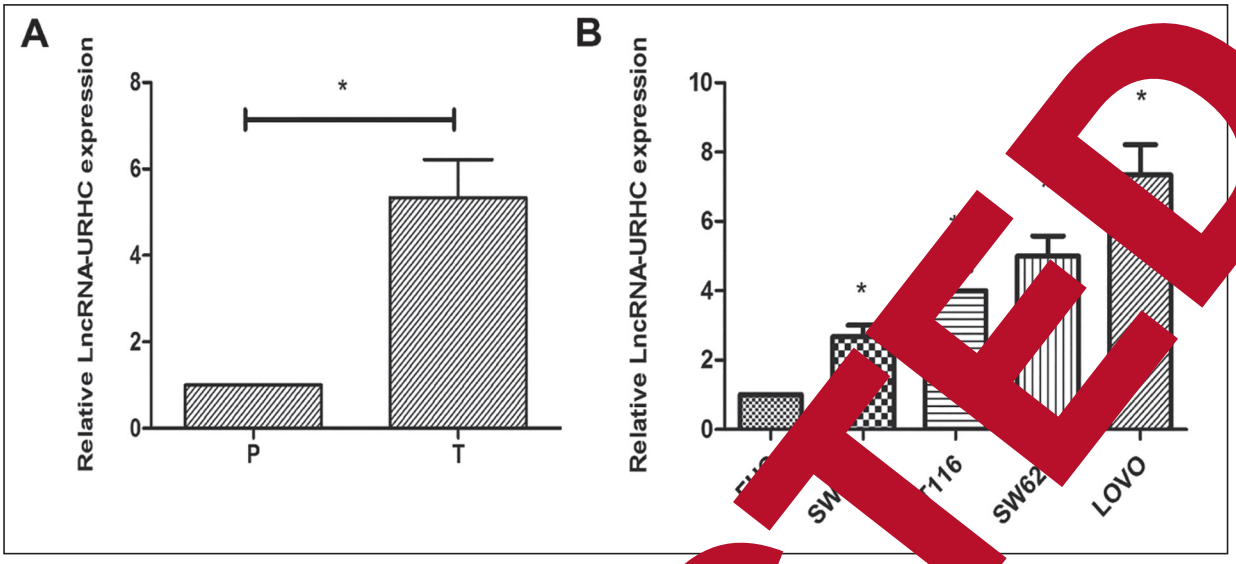


Figure 1. LncRNA-URHC expression was increased in tumor tiss l lines. ***A,*** The rela e expression of lncRNA-URHC was detected by RT-PCR assay. P: adjacent tissues; T: tu issues. ***B,*** The relative expression of lncRNA-URHC was investigated among colorectal cancer cell lines (SW480, HCT SW620, LOVO a normal cell line FHC) by RT-PCR assay. *$p < 0.05$.

Results

LncRNA-URHC Expression in Cancer Samples and Cells Was Significantly Increased

The expression of lncRNA-URHC in colo tal cancer tissues was higher th that in adj cent tissues ($p < 0.05$, Figu ombined with clinical information, fou hat the expression of lncRNA-UR was n ated to the gender and age of th nt, b ly related to tumor si m nd staging (Table I). At the sa me, w ted the expression of lncR RHC in co l cancer cells by qRT-PC sults sugges hat the expr RNA-URHC in colorectal cancer cells was higher than that in human normal lls ($p < 0.05$, Figure 1B). These results suggest NA-URHC plays an essential role in is proliferation and invasion of colorectal cancer.

siRNA Can Inhibit the Expression of LncRNA-URHC in Colorectal Cancer Cells

The expression of lncRNA-URHC was detected by siRNA-lncRNA-URHC, and the interference efficiency was verified by qRT-PCR. Compared with si-NC, the expression of lncRNA-URHC in the cells transfected with siRNA lncRNA-URHC was significantly decreased ($p < 0.05$, Figure 2).

Table I. L A-URHC expressi linical characteristics of patients with colorectal cancer.

Fac		NO.	LncRNA-URHC		p
			High	Low	
Gender	Male	47	26	21	0.908
	Female	30	17	13	
Ag ars)	< 60	36	20	16	0.962
	≥ 60	41	23	18	
Tu	< 5	42	17	25	0.003
	≥ 5	35	26	9	
mph nodes metastasis	Negative	49	22	27	0.011
	Positive	28	21	7	
stage	I-II	31	11	20	0.003
	III-IV	46	32	14	

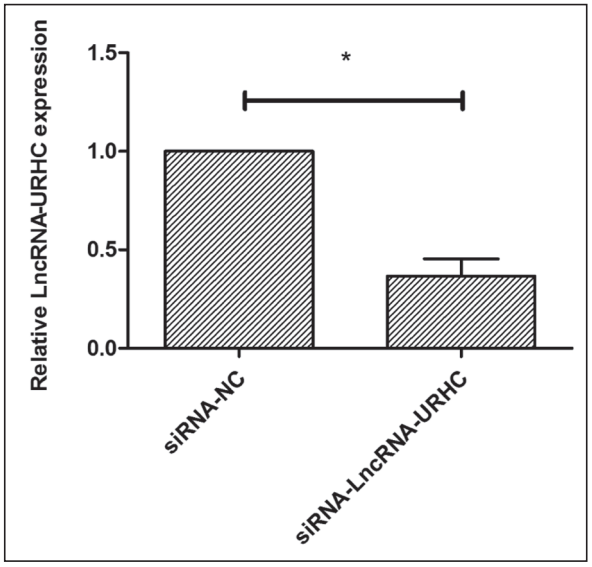


Figure 2. siRNA can inhibit the expression of lncRNA-URHC. The relative expression of lncRNA-URHC was investigated between siRNA-NC (siRNA-negative control) and siRNA-lncRNA-URHC by RT-PCR assay. *$p < 0.05$.

The results showed that siRNA lncRNA-URHC could inhibit the expression of lncRNA-UR in colorectal cancer cells.

Down-regulation of LncRNA-URHC Expression Can Suppress the Proliferation and Promote Apoptosis

SiRNA lncRNA-URHC and re transfected into the cells, and th ability of the cells was detected CK-8 eration assay. The results showed that the proliferation ability of the cells was not significant after transfection for 24 h, and th ability of the cells was significant ifferent after transfection at 48 h and 72 h (5). The proliferation capacity decreased sign in cells with downregulation of ln NA-UR igure 3A). Moreover, cells apo s was eva flow cytometry assay. ve res showed down-regulation of NA-U C expression can promote apopt col tal can cells (Figure 3B).

Down-reg on of Lnc HC Expressi its the Inva on of Colore Can ells

Subsequently, in o detect the effect of lnc RHC on the ion ability of colo cancer cells, transwell invasion assay pre- ed that the nu ber of invasive colorectal cancer cells transfe with siRNA lncRNA-URHC significantl wer than that of transfected si control up. The results showed that dow of lncRNA-URHC could inhibit the invasion of colorectal cancer cells ($p < 0.05$) (Figure 4A and B).

nc A-URHC Regulates the Expression of EMT-related Markers

To further explore whether lncRNA-URHC had an effect on the EMT process, we examined the expression levels of E-cadherin, N-cadherin, vimentin, snail protein by Western blot. The results

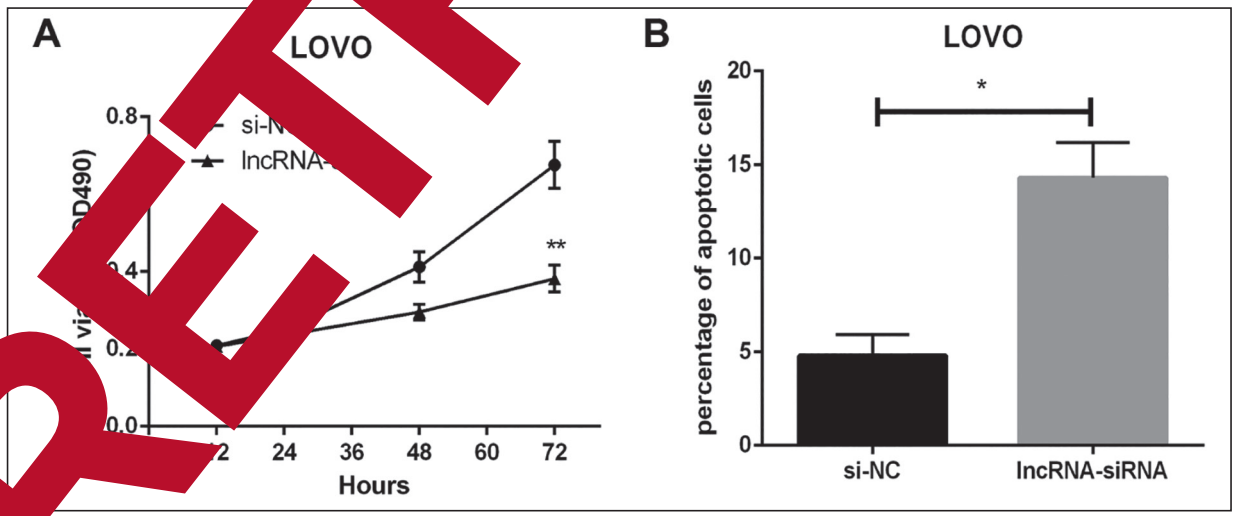


Figure 3. Down-regulation of lncRNA-URHC expression can inhibit cells proliferation and promote cells apoptosis. ***A,*** Cell pr n was detected at 0 h, 24 h, 48 h, 72 h between si-NC and lncRNA-siRNA by CCK-8 assay. ***B,*** Cell apoptosis was detect between si-NC and lncRNA-siRNA by flow cytometry assay. *$p < 0.05$.

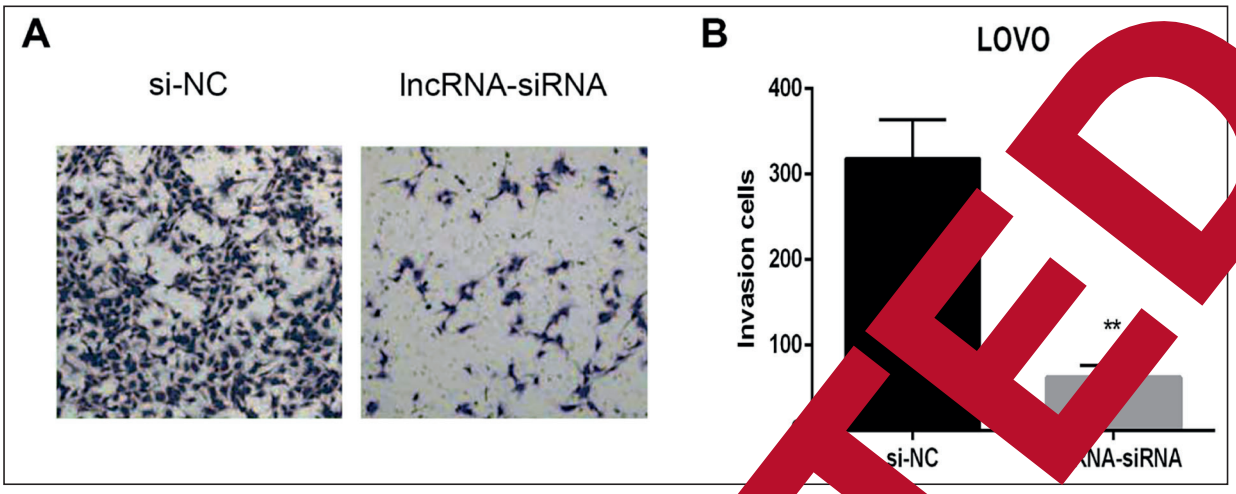


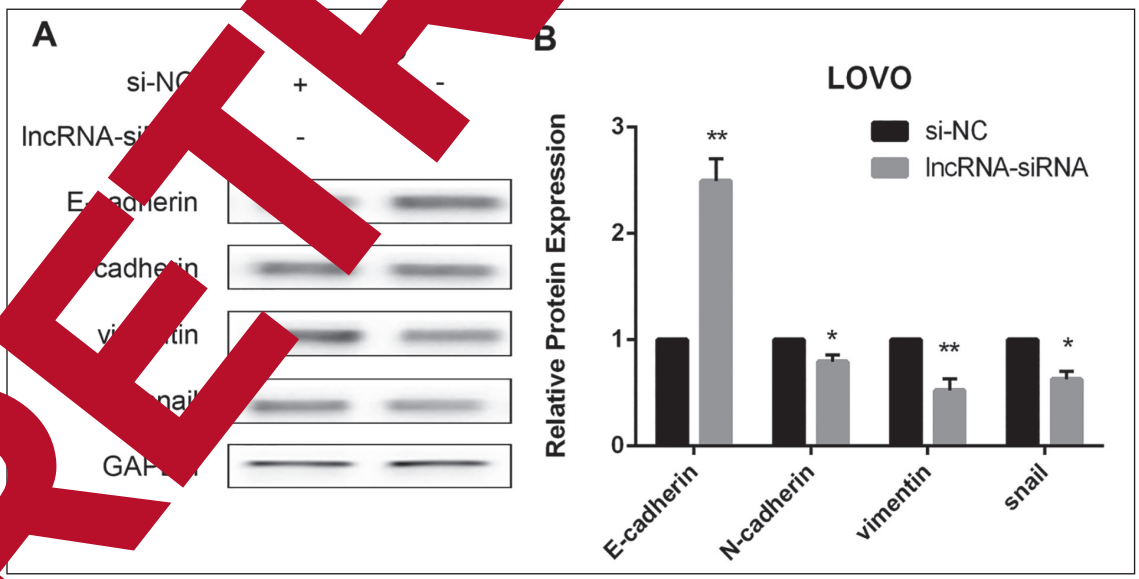
Figure 4. Down-regulation of lncRNA-URHC expression inhibits cells invasion. ***A,*** and ***B,*** Cells invasiveness was explored between si-NC and lncRNA- siRNA through transwell assay. *$p < 0.05$.

showed that down-regulation of lncRNA-URHC could enhance the expression of E-cadherin and reduce the expression of N-cadherin, vimentin and snail (Figure 5A and B). These results suggest that abnormal expression of lncRNA-URHC regulates the expression level of EMT-related markers.

Discussion

The incidence of colorectal cancer is serious, endangering people's life and. From the point of view of molecular le it is v import-

to explore the occurrence and development of orectal cance find out the early diagnosis rognosis, th ognosis evaluation index and th iotherar rget to improve the clinical diag atment of colorectal cancer. In recent years, more and more reports confirmed hat abnormal expression of lncRNAs play an regulatory role in the development of l cancer. For instance, decreased expression of lncRNA BANCR promotes cancer cells proliferation through modulating the expression of p21 gene[11]. MALAT1 expression was increased in colorectal cancer tissues, and might be acted as



5. Abnormal expression of lncRNA-URHC regulates the expression of EMT-related markers. ***A,*** and ***B,*** The relative ex levels of E-cadherin, N-cadherin, vimentin, and snail protein were investigated by Western blot assay. GAPDH was a as the control group. *$p < 0.05$.

a poor prognosis in the patients with stage II/III[12]. Xue et al[13] reported that two lncRNAs (HOTAIR and lncRNA-422) were involved in the progression of human colorectal cancer via genome-wide analysis. The genetic variants of HOTAIR were participated in the risk of patients with colorectal cancer, and rs7958904 might be a predictive marker for diagnosis[14]. LncRNA MALAT1 could enhance tumor cells growth and invasion by bound to SFPQ and releasing PTBP2 from SFPQ/PTBP2 complex[15].

We focused on the role of lncRNA-URHC in the proliferation, apoptosis and invasion of colorectal cancer cells. Firstly, clinical data analysis revealed that lncRNA-URHC expression in cancer tissues was higher than the adjacent normal tissues. lncRNA-URHC was also related to colorectal cancer development process, such as tumor size, metastasis and staging. All these findings indicated that lncRNA-URHC was a cancer-promoting gene. Subsequently, the results of *in vitro* cell experiments indicated the expression of lncRNA-URHC in colorectal cancer cells was higher than that in normal cell. The transfection specific siRNA lncRNA-URHC could reduce the expression of lncRNA-URHC in colorectal cancer cells. We further confirmed that down-regulation of lncRNA-URHC could inhibit cell proliferation and invasion by CCK-8 and transwell experiments. Through flow cytometry assay, we also discovered that down-regulation of lncRNA-URHC could promote cell apoptosis.

EMT acts as an important factor in the process of tumor metastasis. EMT-related markers include E-cadherin, N-cadherin, vimentin, snail and so on. The protein expression of genes involved in EMT process predicts poor prognosis and metastasis in cancer patients. Subsequently, we detected EMT-related markers by Western blot, and found that down-regulation of lncRNA-URHC could elevate the level of E-cadherin and reduce the level of N-cadherin, vimentin and snail.

Conclusions

We showed that down-regulation of lncRNA-URHC can inhibit the proliferation and promote apoptosis of colorectal cancer cells. Moreover, down-regulation of lncRNA-URHC can inhibit cells invasion and EMT process. Our work provides a potential targeted therapy for the clinical treatment of colorectal cancer.

Conflict of Interest

The Authors declare that they have no conflicts of interest.

References

1) Brenner H, Kloor M, Pox CB. Colorectal cancer. Lancet 2014; 383: 1490-1502.
2) Slaby O, Laga R, Sedlacek O. Therapeutic targeting of non-coding RNAs in cancer. Biochem J 2017; 474: 4219-4251.
3) Yang X, Duan Z, [illegible] non-coding RNA FOXD2-AS1 functions as tumor promoter in colorectal cancer by regulating Wnt and Notch signaling pathway. Eur Rev Pharmacol Sci 2017; [illegible]91.
4) McMullen JR, [illegible]. Long non-coding RNAs (lncRNAs) in skeletal and cardiac muscle: potential therapeutic and diagnostic targets? Clin Sci (Lond), 2016; 130: 2245-2256.
5) 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.
6) [illegible]M, Wong CW, Wang Y, Wang WM, [illegible] 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; [illegible]513-22525.
7) Iguchi T, Uchi R, Nambara S, Saito T, Komatsu H, Hirata H, Ueda M, Sakimura S, Takano Y, Kurashige J, Shinden Y, Eguchi H, Sugimachi K, Maehara Y, Mimori K. A long noncoding RNA, lncRNA-ATB, is involved in the progression and prognosis of colorectal cancer. Anticancer Res 2015; 35: 1385-1388.
8) Li L, Zhang L, Zhang Y, Zhou F. Increased expression of lncRNA BANCR is associated with clinical progression and poor prognosis in gastric cancer. Biomed Pharmacother 2015; 72: 109-112.
9) Zhao J, Liu Y, Zhang W, Zhou Z, Wu J, Cui P, Zhang Y, Huang G. Long non-coding RNA Linc00152 is involved in cell cycle arrest, apoptosis, epithelial to mesenchymal transition, cell migration and invasion in gastric cancer. Cell Cycle 2015; 14: 3112-3123.
10) Gigic B, Boeing H, Toth R, Bohm J, Habermann N, Scherer D, Schrotz-King P, Abbenhardt-Martin C, Skender S, Brenner H, Chang-Claude J, Hoffmeister M, Syrjala K, Jacobsen PB, Schneider M, Ulrich A, Ulrich CM. Associations between dietary patterns and longitudinal quality of life changes in colorectal cancer patients: the ColoCare study. Nutr Cancer 2018; 70: 51-60.
11) Shi Y, Liu Y, Wang J, Jie D, Yun T, Li W, Yan L, Wang K, Feng J. Downregulated long noncoding RNA BANCR promotes the proliferation of colorectal cancer cells via downregualtion of p21 expression. PLoS One 2015; 10: e122679.

12) 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.

13) 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.

14) 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.

15) Ji Q, Zhang L, Liu X, Zhou L, Wang W, Han Z, Sui H, Tang Y, Wang Y, Liu N, Ren J, Hou F, Li Q. Long non-coding RNA MALAT1 promotes tumour growth and metastasis in colorectal cancer through binding to SFPQ and releasing oncogene PTBP2 from SFPQ/PTBP2 complex. Br J Cancer 2014; 111: 736-748.

16) Taube JH, Malouf GG, Lu E, Sphyris N, Vijay V, Ramachandran PP, Ueno KR, Gaur S, Nicolo[illegible]si S, Herschkowitz JI, Rosen JM, Issa[illegible] Chang JT, Mani SA. Epigenetic [illegible]cing of [illegible]croRNA-203 is required for EMT[illegible] cancer stem cell properties. Sci Rep 2013[illegible]7.

17) Kaneko N, Kawano S, Yasuda K, H[illegible] Y, Sakamoto T, Matsubara R, Got[illegible]unno T, [illegible] Morioka M, Hattori T, Tan[illegible] Tanaka H, K[illegible] Nakamura S. Differe[illegible]oles of kallikrein ed peptidase 6 in [illegible]gnant t[illegible]ormation and DeltaNp63beta-r[illegible]ted [illegible]elial-mesenchymal transition of [illegible]s cell c[illegible]oma. Oral Oncol 2[illegible]75.

18) Takai M, T[illegible]Y, Kawagu[illegible]A[illegible]a K, Fujiwara S, [illegible] T, Tsunetoh S[illegible]Y, Sasaki H, Kanem[illegible]e A, Ohmichi [illegible]The EMT (epithel[illegible]ese[illegible]l-transition)-related protein expre[illegible]ion indic[illegible]e metastatic status and prognosis in patient[illegible]varian cancer. J Ovar[illegible]s 2014; 7: 76.