European Review for Medical and Pharmacological Sciences 2019; 23: 6453-6458

Knockdown of long noncoding RNA linc-ITGB1 inhibits tumor metastasis in colorectal cancer through suppressing BDNF

W.-B. WAN[1], Q.-L. KONG[2]

[1]Department of Gastrointestinal Surgery, Heze Municipal Hospital, Heze, Ch
[2]Department of Oncology, Affiliated Hospital of Jining Medical College, J g, Chin

Abstract. – OBJECTIVE: Recently, long noncoding RNA (lncRNAs) have got much attention for their role in the progression of cancers. In this research, lncRNA linc-ITGB1 was studied to identify whether it affects the development of colorectal cancer (CRC).

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was performed to detect the linc-ITGB1 expression of CRC cells and tissues. Moreover, the associations between linc-ITGB1 expression level and patients' overall survival rate we ther analyzed. Furthermore, we conducte tion assays, including wound healing assa d transwell assay, to explore the effect of ITGB1 on CRC metastasis *in vitro*. In additi the underlying mechanism was f r studie

RESULTS: RT-qPCR results hat linc ITGB1 expression level was her i C samples than that in adjace sues. -ITGB1 expression was related to p all survival time. Mo ve ation d cell invasion were i ted a -ITGB1 was silenced *in vitro*. dition, the A and protein expression F was dow ated by the silence o Furthermo , the expression lev BDN higher in CRC samples than in adjacent es and was positively re d to the express linc-ITGB.

CO USION These results indicate that linc B1 co enhance CRC cell migration and ion upreg ting BDNF. Linc-ITGB1 might ential apeutic target for CRC nts.

Key rds:
l noncoding RNA, Linc-ITGB1, Colorectal cancer

Introduction

Colorectal cancer (CRC) is the third most prevalently diagnosed malignancy worldwide. Though t ity of CRC s been decreased the p cades, CRC is still the fourth leading caus cancer-related deaths glo early 1.4 m cases were newly d osed of CRC in 2012, and 693,900 CRC nts died i e same year[2]. Most of the nts have d oped metastasis when they a st diagn . Despite the technological adv en made in screening tests and th e management, the overall survival rate of CRC patients with advanced stage unsatisfied[3]. Therefore, it's urgent to the underlying molecular mechanisms of CRC tumorigenesis and figure out new therapeutic targets to improve the poor prognosis.

Non-coding RNAs (ncRNAs) account for 99% of total transcribed RNAs. Long noncoding RNAs (lncRNAs), as a subtype of ncRNAs, are longer than 200 nucleotides. LncRNAs are key regulators in numerous biological processes, especially in the progression and development of cancers. For example, lncRNA ATB promotes cell migration and cell invasion in glioma through suppressing the expression of microRNA 2043p[4]. By modulating SF1 and suppressing expression level of miR-184, lncRNA UCA1 accelerates cell proliferation and cisplatin resistance in oral squamous cell carcinoma[5]. LncRNA RUNX1-IT1 acts as a tumor suppressor in colorectal cancer by the inhibition of cell migration and cell proliferation[6]. Through targeting miR-221/SOCS3, lncRNA GAS5 suppresses cell proliferation, cell metastasis, and gemcitabine resistance in pancreatic cancer[7]. However, the function of linc-ITGB1 in CRC has not been explored so far.

In this study, we found out that the expression of linc-ITGB1 was remarkably higher in CRC tissues. Moreover, linc-ITGB1 promoted the mi-

Corresponding Author: Qingli Kong, MM; e-mail: 1652577449@qq.com

gration and invasion of CRC cell *in vitro*. Moreover, our further work explored the underlying mechanism of how linc-ITGB1 functioned in CRC development.

Patients and Methods

Cell Lines and Clinical Samples

65 CRC patients were enrolled for CRC tissues which undergo Gastrointestinal Surgery in Heze Municiple Hospital. Written informed consent was achieved before the operation. No radiotherapy or chemotherapy was performed before the surgery. All tissues were kept at –80°C. All tissues were analyzed by an experienced pathologist. The protocol of the study was approved by the Ethics Committee of Heze Municiple Hospital.

Cell Culture

Human renal cancer cell HCT116, SW620, and SW480 and normal human colonic epithelial cells (NCM460) were offered by Chinese Type Culture Collection, Chinese Academy of Sci[illegible] (Shanghai, China). Culture medium cons[illegible] 10% fetal bovine serum (FBS; Gibco, Roc[illegible]e, MD, USA), Dulbecco's Modified Eagle's Me[illegible] (DMEM; Gibco, Rockville, MD, USA) and pe[illegible]cillin. Cells were cultured at 37°[illegible] humidifie[illegible] incubator containing 5% CO[illegible]

Cell Transfection

We purchased lenti[illegible]us [illegible] pin RNA (shRNA[illegible]gainst [illegible]TGB1 from GenePharma (Sh[illegible]ai, China). [illegible]fectamine 2000 (Invitrog[illegible]ad, CA, US[illegible]as used for the transf[illegible]on o[illegible] cells. 48 h later, Real Time qu[illegible]titative-Poly[illegible]e Chain Reaction (RT-qPC[illegible] was used to de[illegible] linc-ITGB1 expressi[illegible]vel in[illegible]ese cells.

RN[illegible]n an[illegible]T-qPCR

Acc[illegible] to th[illegible]anufacturer's protocol, [illegible]l rea[illegible]gen, Carlsbad, CA, USA) wa[illegible]lized t[illegible]rate the total RNA. Through rev[illegible] Transcri[illegible]ion Kit (TaKaRa Biotechnolog[illegible]alian, China), the total RNA was [illegible]erse-tra[illegible]ribed to complementary deoxyri[illegible]e nucleic acids (cDNAs). Linc-ITGB1, for[illegible] 5′-CCTCTCAGCCTCCAGCGTTG-3′ and rev[illegible]e 5′-TGCTCTTGCTCACTCACACTCC-3′; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (internal control), forward 5′-ATGTCTTTCCGTGTTCCTACTGT-3′ and reverse 5′-TTTCCCTCAGACTCCTCCTTG-3′. The relative expression was calculated by performing the $2^{-\Delta\Delta CT}$ method. Thermal cycle was as f[illegible] sec at 95°C, 5 sec for 40 cycles at [illegible], 35 s[illegible] at 60°C.

Western Blot Analysis

Reagent radioimmunop[illegible]itation a[illegible]RI[illegible] PA; Beyotime, Shangh[illegible]hina) was u[illegible] extract protein from [illegible]. Qua[illegible]ing pro[illegible] concentrations was [illegible]med [illegible]bicinc[illegible]ninic acid (BCA) prote[illegible]ass [illegible]aKaR[illegible]alian, China). Sodiu[illegible]odecy[illegible]phat[illegible]yacrylamide Gel [illegible]rophoresis [illegible]GE) was utilized to [illegible]he target pr[illegible]s. After replaced to [illegible] poly[illegible]idene difluoride (PVDF) membrane (Millipo[illegible]illerica, MA, USA), the[illegible] incubated [illegible] antibodies. Rabbit a[illegible]GAPDH and rabbit anti-BDNF, as well as [illegible] anti-rabbit [illegible]ondary antibody, were pro[illegible]d by Abcam [illegible]mbridge, MA, USA). Image J [illegible]ware was [illegible]plied for the assessment of prot[illegible].

Wound Healing Assay

[illegible] transferred into 6-well plates, cells were [illegible] in DMEM medium overnight. Then, they were scratched by a sterile 10 µL pipette tip and were cultured in serum-free DMEM. Each assay was repeated in triplicate independently. Wound closure was viewed at 0 and 24 h.

Transwell Assay

5×10^4 cells in 200 µL serum-free DMEM were transformed to top chamber of an 8 µm pore size insert (Corning, Corning, NY, USA) coated with 50 µg transwell (BD, Bedford, MA, USA). DMEM and FBS were added to the bottom chamber. 48 h later, the top surface of chambers was immersed for 10 min with precooling methanol after wiped by cotton swab. Next, they were stained in crystal violet for 30 min. The data for invasion membrane was counted in three fields.

Statistical Analysis

Statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 20.0 (SPSS, Chicago, IL, USA). Independent-sample test and Kaplan-Meier method were performed for analysis of the data. $p<0.05$ was considered statistically significant.

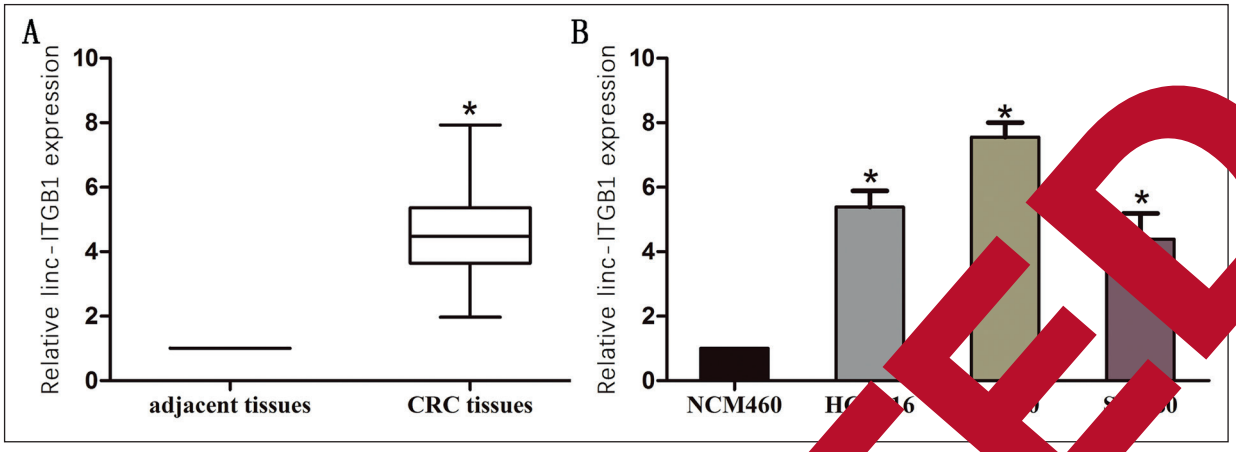

Figure 1. Expression levels of linc-ITGB1 were increased in CRC tissues a[illegible]es. ***A,*** Linc-I[illegible]pression was significantly increased in the CRC tissues compared with adjacent tissues. [illegible] levels of linc-[illegible]GB1 relative to GAPDH were determined in the human CRC cell lines and normal human c[illegible]nic epit[illegible]lls (NCM460) by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

Results

Linc-ITGB1 Expression Level in CRC Tissues and Cells

Firstly, linc-ITGB1 expression was detect[illegible] RT-qPCR in 65 patients' tissues and 3 C[illegible] lines. As a result, linc-ITGB1 was signifi[illegible]ly upregulated in CRC tissue samples (Figure [illegible] The linc-ITGB1 expression level of CRC c[illegible] was higher than that of NCM46[illegible]re 1B).

High Expression of Lin[illegible]GB1 [illegible]s Correlated With Ove[illegible] Surviv[illegible] CRC Patients

Patients' survival [illegible]er sur[illegible]as analyzed through the Kap[illegible]Meier meth[illegible]y method of average lin[illegible] expression[illegible] divided 65 CRC [illegible]ients [illegible] two groups, high-linc-ITGB[illegible]nd low-lin[illegible]B1. Kaplan-Meier analysis [illegible]wed that CRC [illegible]ts in the high-linc-I[illegible] grou[illegible]had a poore[illegible] overall survival wh[illegible]mpar[illegible]th the low-linc-ITGB1 group (Fig[illegible]

[illegible]kdo[illegible]f [illegible]-ITGB1 Inhibited Cell M[illegible]tion a[illegible]vasion in CRC Cells

I[illegible]is study, [illegible]e chose SW620 CRC cell lines f[illegible]own of linc-ITGB1. Then, linc-[illegible]B1 expression was detected by RT-qPCR [illegible]gure 3A). Moreover, the results of wound [illegible] assay showed that knockdown of linc-IT[illegible] inhibited the migrated ability of SW620 cells (Figure 3B). The outcome of the transwell assay also revealed that the number of migrated [illegible] and invade[illegible]lls was remarkably decreased [illegible] that linc-IT[illegible]l was silenced in CRC cells ([illegible] 3C and [illegible]

The In[illegible]on Between BDNF and Linc-ITGB1 in CRC

[illegible]PCR results showed that the expression [illegible]BDNF in CRC cells was significantly low-er in linc-ITGB1 shRNA (sh-linc-ITGB1) group when compared with the BDNF level in empty vector (control) group (Figure 4A). The result of Western blot assay showed that after linc-ITGB1 was silenced, BDNF could be downregulated at the protein level (Figure 4B). Furthermore, we found that BDNF expression in CRC tissues was

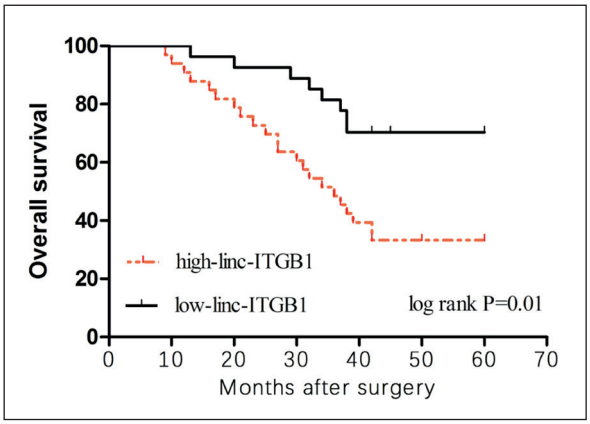

Figure 2. Expression levels of linc-ITGB1 was associated with poorer overall survival of CRC patients. High level of linc-ITGB1 was associated with poorer overall survival of CRC patients. *$p<0.05$.

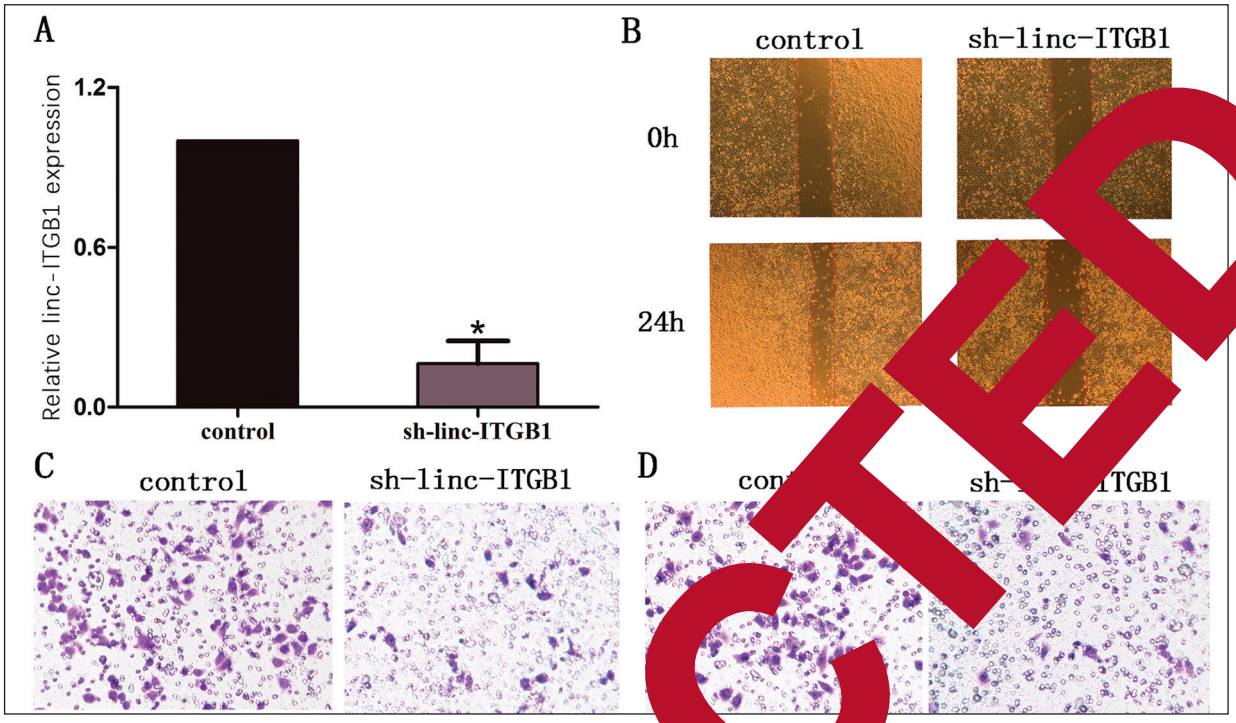

Figure 3. Silenced of linc-ITGB1 inhibited CRC cell migration a[illegible] Linc-ITGB1 expression in CRC cells transduced with linc-ITGB1 shRNA (sh-linc-ITGB[illegible] the empty ve[illegible]) was detected by RT-qPCR. GAPDH was used as an internal control. ***B,*** Wound healin[illegible] that the silence of linc-ITGB1 significantly repressed cell migration in CRC cells. ***C,*** Transwell assay showed [illegible] grated cells was significantly decreased *via* silence of linc-ITGB1 in CRC cells. ***D,*** Transwell assay showed [illegible] the nu[illegible]ed cells was significantly decreased *via* silence of linc-ITGB1 in CRC cells. The results represent the a[illegible] ee ind[illegible]pendent experiments (mean ± standard error of the mean). *$p<0.05$.

remarkably higher when c[illegible]ared v[illegible] that of adjacent tissues (Figure [illegible] Correla[illegible]sis demonstrated that the [illegible] positively correlate[illegible] the [illegible]GB1 expression in CRC tissu[illegible]igure 4D).

Discu[illegible]

A l[illegible] numb[illegible] of evidence [illegible]as suggested that lnc[illegible]s are c[illegible]al regulators in carcinogenesis of C[illegible] Fo[illegible]stance [illegible]ough SIRT1 mediated autopha[illegible]RNA [illegible] promotes 5-Fu resistance [illegible]orect[illegible] sponging to miR-194-5p[8]. Th[illegible]h regu[illegible] of the miR-1254-WNT11 signal[illegible]hway, ln[illegible]NA ABHD11-AS1 facilitates c[illegible]n and cell invasion in CRC, which [illegible] provide a new therapeutic strategy for CRC[9]. [illegible]regulation of lncRNA LINC01510 is negative-[illegible]ociated with the prognosis in patients with CR[illegible] which may offer a potential independent prognostic biomarker[10]. LncRNA TP73AS1 markedly promotes cell apoptosis and depresses cell proliferation in colorectal cancer by functioning as a competing endogenous RNA for miR103 to modulate the expression of PTEN[11]. In addition, lncRNA ENST00000547547 acts as a tumor suppressor in CRC by inhibiting cell proliferation, cell invasion, and cell metastasis in CRC[12].

Integrin subunit β1 (ITGB1; linc-ITGB1) has recently been reported to function as a novel oncogene in tumorigenesis. For instance, through reducing the expression level of Snail, the silence of linc-ITGB1 represses epithelial-mesenchymal transition and cancer stemness in non-small cell lung cancer[13]. LncRNA linc-ITGB1 facilitates the proliferation, invasion, and migration of human hepatoma carcinoma cell *via* upregulation of ROCK1[14]. By targeting ITGB1, miR-183-5p depresses cell migration and cell invasion in cervical cancer[15]. In addition, migration and invasion of the gallbladder cancer cell are found to be remarkably inhibited when linc-ITGB1 is knocked down[16]. We found that linc-ITGB1 was upregulated both in CRC samples and cells. Besides, a close relationship was seen between patients' prognosis and

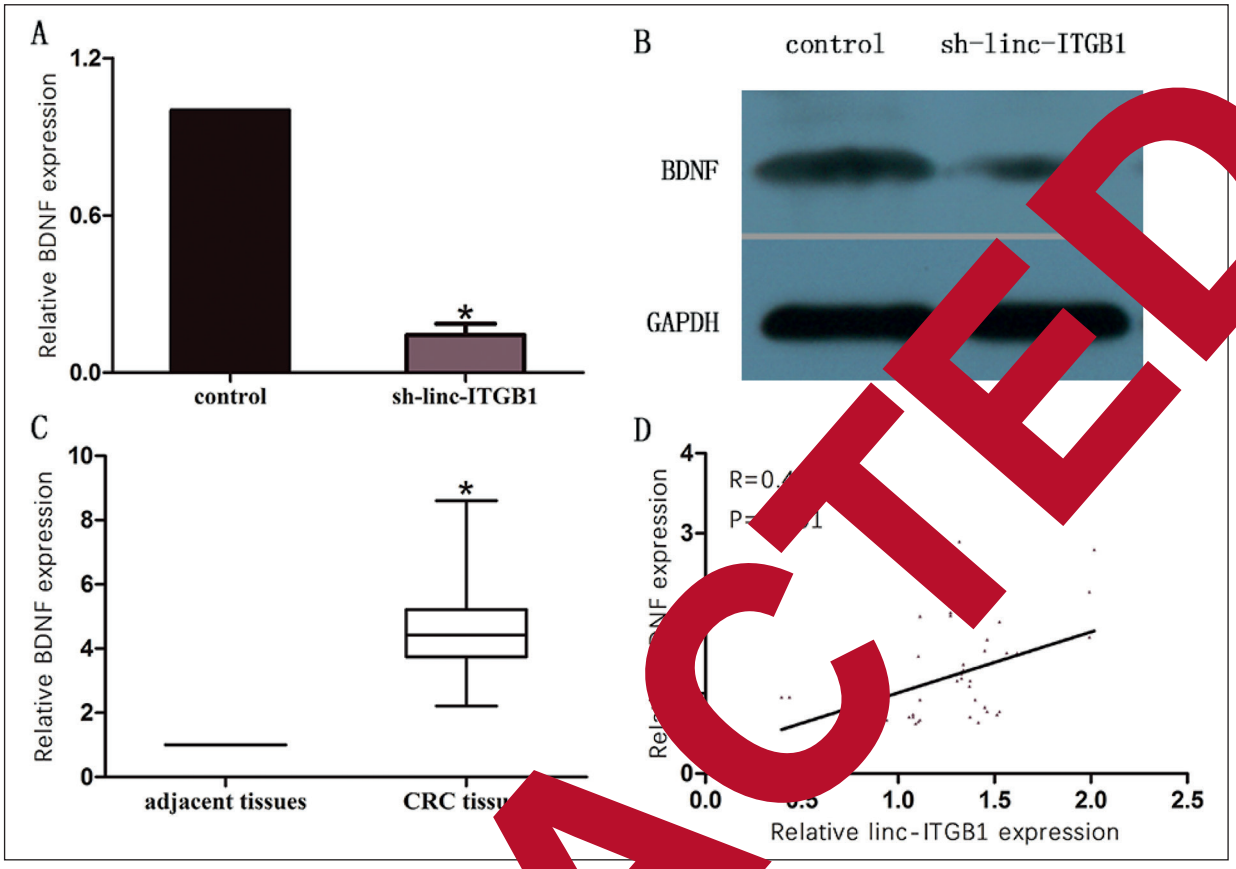

Figure 4. Interaction between linc-ITGB1 and BDNF. [illegible] results showed that the BDNF expression was decreased in linc-ITGB1 shRNA (sh-linc-ITGB1) compared with the [illegible]ector (control). ***B,*** Western blot assay revealed that the BDNF protein expression was decreased in li[illegible] shRNA (s[illegible]-ITGB1) compared with the empty vector (control). ***C,*** BDNF was significantly upregulated in CR[illegible]ared with [illegible]ent tissues. ***D,*** The linear correlation between the expression level of BDNF and linc-ITGB1 in [illegible] tissue[illegible] e results r[illegible]ent the average of three independent experiments. Data are presented as the mean ± standa[illegible]or of the [illegible] $^*p<0.05$.

expression level of [illegible]TGB[illegible]ermore, the silence of linc-IT[illegible] suppresse[illegible] migration and invasion in [illegible]. Above res[illegible]dicated that linc-ITG[illegible] prom[illegible] the tumorigenesis of CRC and m[illegible]ht act as an [illegible]ene.

Brain[illegible]ved neurotrop[illegible]tor (BDNF) is widel[illegible]pressed in the mamm[illegible]lian brain which is a [illegible]mber [illegible]he neurotrophic superfamily. Som[illegible]ve ind[illegible]ed that BDNF plays a crucia[illegible] tum[illegible]owth and metastasis of can[illegible]ding to its receptors. For ins[illegible]e, BD[illegible]ys an important role in the pro[illegible]sion of [illegible]arian cancer by inducing cell m[illegible] invasion in ovarian cancer[17]. The [illegible]rexpression of BDNF could effectively reverse [illegible] functions of miR-15a-5p on cell proliferation [illegible]atocellular carcinoma[18]. Through inhibition of [illegible]NF, miR-497 functions as a tumor suppressor in thyroid cancer by depressing tumor growth and cell invasion[19]. In addition, dual inhibition of autophagy and BDNF/TrkB may offer a potential therapeutic target for CRC[20]. Our study showed that the expression level of BDNF could be downregulated after knockdown of linc-ITGB1. Furthermore, the BDNF expression in CRC tissues was positively related to the linc-ITGB1 expression. All the results above suggested that linc-ITGB1 might promote tumorigenesis of CRC by targeting BDNF.

Conclusions

We found that linc-ITGB1 was remarkably upregulated and was negatively related to the overall survival of CRC patients. Besides, linc-ITGB1 could facilitate cell migration and invasion in CRC through targeting BDNF. These findings suggest that linc-ITGB1 may contribute to therapy for CRC as a candidate target.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Miao Z, Wang K, Wang X, Zhang C, Xu Y. TNF-alpha-308G/A polymorphism and the risk of colorectal cancer: a systematic review and an updated meta-analysis. J BUON 2018; 23: 1616-1624.
2) Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-E386.
3) Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, Inamura K, Kim SA, Kuchiba A, Yamauchi M, Imamura Y, Willett WC, Rosner BA, Fuchs CS, Giovannucci E, Ogino S, Chan AT. Long-term colorectal-cancer incidence and mortality after lower endoscopy. N Engl J Med 2013; 369: 1095-1105.
4) Bian EB, Chen EF, Xu YD, Yang ZH, Tang F, Ma CC, Wang HL, Zhao B. Exosomal lncRNAATB activates astrocytes that promote glioma cell invasion. Int J Oncol 2019; 54: 713-721.
5) Fang Z, Zhao J, Xie W, Sun Q, Wang H, Qiao [illegible] lncRNA UCA1 promotes proliferation and [illegible]tin resistance of oral squamous cell ca[illegible] by suppressing miR-184 expression. Cance[illegible]d 2017; 6: 2897-2908.
6) Shi J, Zhong X, Song Y, Wu Z, Gao P, Zhao J, [illegible] J, Wang J, Liu J, Wang Z. Long [illegible]oding RN[illegible] RUNX1-IT1 plays a tumor[illegible]e role i[illegible] colorectal cancer by inhi[illegible]g cell [illegible]iferation and migration. Cell Bio[illegible] Funct [illegible]: 37: 11-20.
7) Liu B, Wu S, Ma J, [illegible] L, Zhang [illegible], Shang M, Mao A[illegible]RNA [illegible]everses EMT and tumor st[illegible]ell-mediated [illegible]itabine resistance an[illegible]asis by targ[illegible]miR-221/SOCS3 in[illegible]cre[illegible]ancer. Mol [illegible]er Nucleic Acids 2018; 13: 472-[illegible]
8) Wang [illegible]ian D, Yuan Z, [illegible] Zhao Z, Yang R, Jin Y, [illegible], Chen Y, Wang G, [illegible]X, Wang X. Long n[illegible]oding [illegible] H19 confers 5-Fu resistance in [illegible]rectal [illegible]er by promoting SIRT1-mediated [illegible]ell Dea[illegible]is 2018; 9: 1149.
9) He [illegible]Z, Liu [illegible]g X, Chen L, Han H. Long [illegible]onco[illegible]RNA [illegible]D11-AS1 promote cells pro[illegible]ration[illegible]sion of colorectal cancer via [illegible]ulating [illegible]iR-1254-WNT11 pathway. J Cell [illegible]siol 2018 Dec 7. doi: 10.1002/jcp.27877. [Epub [illegible]nt].
10) Zhou JF, Wang HG, Ma TH, Yan W, Wu SN, Shi YT, Yang XZ. Long noncoding RNA LINC01510 is highly expressed in colorectal cancer and predicts favorable prognosis. Eur Rev Med [illegible]col Sci 2018; 22: 7710-7715.
11) Jia Z, Peng J, Yang Z, Chen J, Liu [illegible] D, He [illegible] Long non-coding RNA TP73AS[illegible]omotes colorectal cancer proliferation by a[illegible] a ceRNA for miR103 to regulate PTEN exp[illegible] Gene 2019; 685: 222-229.
12) Ai X, Lei Q, Shen X, Li [illegible] C, Xie G, Lu [illegible] W, Hu G, Wu H, Chen [illegible] X, Lin [illegible] Long no[illegible]ing RNA ENST000[illegible]7547 i[illegible]s cell prol[illegible]ation, invasion an[illegible]tion[illegible]olorect[illegible]ancer cells. Oncol R[illegible]201[illegible]491.
13) Guo L, Sun[illegible]u S, Xu [illegible]g C[illegible]ang L, Li W, Wang [illegible]g G, Guo [illegible]wn of long non-co[illegible]inc-ITGB1 in[illegible] cancer stemness [illegible] ep[illegible] mesenchyma[illegible] transition by reducing the exp[illegible] of Snail in non-small cell lu[illegible]cer. Thorac[illegible]r 2019; 10: 128-136.
14) [illegible] L, Li X, Gao W[illegible]ong non-coding RNA [illegible]inc-ITGB1 promotes cell proliferation, migration, and inv[illegible] in human hepatoma carcinoma by up-regula[illegible] ROCK1. Biosci Rep 2018; 38: [illegible]SR2018128[illegible]
15) [illegible] W, Z[illegible] M, Liu L, Jin D, Wang P, Hu J. Mi[illegible]p inhibits aggressiveness of cervical cancer cells by targeting integrin subunit beta 1 (ITGB1). Med Sci Monit 2018; 24: 7137-7145.
16) [illegible] L, Zhang Y, Lv W, Lu J, Mu J, Liu Y, Dong P. L[illegible]g non-coding RNA Linc-ITGB1 knockdown inhibits cell migration and invasion in GBC-SD/M and GBC-SD gallbladder cancer cell lines. Chem Biol Drug Des 2015; 86: 1064-1071.
17) Lim WC, Kim H, Kim YJ, Park SH, Song JH, Lee KH, Lee IH, Lee YK, So KA, Choi KC, Ko H. Delphinidin inhibits BDNF-induced migration and invasion in SKOV3 ovarian cancer cells. Bioorg Med Chem Lett 2017; 27: 5337-5343.
18) Long J, Jiang C, Liu B, Fang S, Kuang M. MicroRNA-15a-5p suppresses cancer proliferation and division in human hepatocellular carcinoma by targeting BDNF. Tumour Biol 2016; 37: 5821-5828.
19) Wang P, Meng X, Huang Y, Lv Z, Liu J, Wang G, Meng W, Xue S, Zhang Q, Zhang P, Chen G. MicroRNA-497 inhibits thyroid cancer tumor growth and invasion by suppressing BDNF. Oncotarget 2017; 8: 2825-2834.
20) Mazouffre C, Geyl S, Perraud A, Blondy S, Jauberteau MO, Mathonnet M, Verdier M. Dual inhibition of BDNF/TrkB and autophagy: a promising therapeutic approach for colorectal cancer. J Cell Mol Med 2017; 21: 2610-2622.