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LINC00052 inhibits tumor growth, invasion and metastasis by repressing STAT3 in cervical carcinoma

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Abstract. – OBJECTIVE: The vital role of long noncoding RNAs (lncRNAs) in tumor progression has been identified in numerous studies. In this research, the biological function of lncRNA LINC00052 during the development of cervical cancer was mainly explored.

PATIENTS AND METHODS: LINC00052 expression was detected by quantitative Real-time polymerase chain reaction (qRT-PCR) in cervical cancer tissue samples and cell lines. Moreover, the correlation between LINC00052 expression level and disease-free survival rate of cervical cancer patients was analyzed. *In vitro* functions of LINC00052 in cervical cancer were evaluated by proliferation assay, scratch healing assay and transwell assay. In addition, qRT-PCR and Western blot were utilized to explore the underlying mechanism of LINC00052 in the progression of cervical cancer.

RESULTS: LINC00052 expression level was lower in cervical cancer samples than that in adjacent tissues, which was correlated with disease-free survival time. Moreover, cell proliferation, migration and invasion were inhibited through overexpression of LINC00052 in vitro. The mRNA and protein expression of signal transducers and activators of transcription 3 (STAT3) was downregulated after overexpressing LINC00052 in cervical cancer cells. The STAT3 expression level was negatively correlated with the expression of LINC00052 in cervical cancer tissues.

CONCLUSIONS: LINC00052 could repress the metastasis and invasion of cervical cancer cell via suppressing STAT3. LINC00052 might be a novel suppressor in cervical cancer.

Key Words:
Long noncoding RNA, LINC00052, STAT3, Cervical cancer.

Introduction

Cervical cancer is the fourth most common gynecologic malignancy globally, which is also the most prevalent cancer in Chinese women. There were 528,000 diagnosed cases and approximately 266,000 death cases of cervical cancer in 2012[1]. In developing countries, the morbidity accounts for 85% of cervical cancer cases, resulting in more than 200,000 female deaths annually[2,3]. Conventional therapeutic strategies for cervical cancer include surgery, radiotherapy and chemotherapy. Though the cure rate of cervical cancer reaches 90% in early stage, the prognosis of metastatic cervical cancer is very poor. Therefore, it is urgent to clarify the underlying molecular mechanism and develop a new treatment strategy for cervical cancer. Long non-coding RNAs (lncRNAs) are one subtype of non-coding RNAs, which are longer than 200 nucleotides in length. Recently, lncRNAs are widely explored in a variety of biological behaviors. Moreover, evidence proved that lncRNAs serve as an important regulator in the progression of malignant tumors. For example, lncRNA CCAT2 promotes proliferation and metastasis of intrahepatic cholangiocarcinoma, which predicts a poor prognosis of these patients[4]. LncRNA OR3A4 is upregulated in breast cancer and may be a potential therapeutic target and prognostic marker[5]. Interacted with miR-124, lncRNA XIST functions as an oncogene, which promotes cell growth, migration and invasion in bladder cancer[6]. LncRNA SNHG7 promotes cell proliferation and cycle progression in cervical cancer through miR-503/Cyclin D1 pathway[7]. In addition, lncRNA 91H exerts oncogenic properties by up-regulating expression of H19/IGF2, which increases aggres-

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sive phenotype of breast cancer cells[8]. However, the clinical role and biological mechanisms of lncRNA LINC00052 in the development of cervical cancer remain unexplored. In this study, we found out that the expression of LINC00052 was downregulated in cervical cancer. Moreover, LINC00052 inhibited the proliferation, migration and invasion of cervical cancer cells *in vitro*. Furthermore, we explored the underlying mechanism of LINC00052 in mediating the development of cervical cancer.

Patients and Methods

Cell Lines and Clinical Samples

Cervical cancer tissues and adjacent normal tissues were harvested from 60 cervical cancer patients undergoing surgery at Affiliated Hospital of Guilin Medical University. Tissues samples were immediately preserved at –80°C. Before operation, written informed consent was achieved. This study conformed as the Ethics Committee of Affiliated Hospital of Guilin Medical University required. Human cervical cancer cell lines SiHa, HeLa, C4-1 and [illegible] and normal cervical epithelium cell line [illegible] were offered by Chinese Academy of S[illegible]ce (Shanghai, China). Cells were cultured in [illegible] well Park Memorial Institute-1640 (RPMI-16[illegible] (HyClone, South Logan, UT, USA) consisted [illegible] 10% fetal bovine serum (FBS, [illegible]ockville, MD, USA) and 1% penicilli[illegible]sides[illegible]ls were maintained in an incubato[illegible]h 5% C[illegible]at 37°C.

Cell Transfection

Lentiviral virus ta[illegible]ng LI[illegible]52 was compounded and inse[illegible]nto the pLen[illegible]-EGFP-F2A-Puro vecto[illegible]tia Inc., San [illegible]go, CA, USA). Empty [illegible]tor a[illegible] LINC00052 lentiviruses (LINC00052) were p[illegible]ed in 293T cells.

RNA E[illegible]action and Quantitative Rea[illegible]e Po[illegible]erase Chain Reaction (q[illegible]CR)

The[illegible]A was [illegible]acted using TRIzol re-[illegible]ent (In[illegible]a, Ca[illegible]d, CA, USA) and reverse-[illegible]cribed [illegible]mentary deoxyribose nucleic ac[illegible]cDNAs) [illegible]g the reverse Transcription Kit (Tak[illegible] Biotechnology Co., Ltd., Dalian, China). Fo[illegible]the primers using for qRT-PCR: [illegible]C00052, forward 5′-CCTATCCCTTTCTCTA-[illegible]A-3′ and reverse 5′-ACTTCTGCAAAAAC-[illegible]G-3′; MTA1, forward 5′-GCTCTAGAACT-GG[illegible]TTGTTGACATTTCTG-3′ and reverse 5′- GCTCTAGACAAGAACTTGAAGATGTG-CCATT-3′; β-actin, forward 5′- GATC[illegible] GTCAGAGGCT-3′ and reverse [illegible]GCA[illegible] TAGTTGGAAATGC-3′. Therma[illegible]cle was as follows: 30 s at 95°C, 5 s at 95°C[illegible]35 s at 60°C, for a total of 40 cycles.

Western Blot Analysi[illegible]

Reagent radioim[illegible]precip[illegible]tion [illegible] (RIPA) (Beyotime, [illegible]nghai, [illegible]na) was utilized to extract pro[illegible]m[illegible]. Bicin[illegible]inic acid (BCA) pro[illegible] as[illegible] (TaKaR[illegible]Dalian, China) was c[illegible]d for qu[illegible]ing [illegible]tein concentrations. [illegible] target prote[illegible] separated by sodiu[illegible] sulphate-poly[illegible]rylamide gel electrop[illegible]sis (S[illegible]AGE). Next, they were incubated with primar[illegible]odies after loading on the [illegible]ylidene diflu[illegible]PVDF) membranes ([illegible]pore, Billerica, MA, USA). Cell Signaling [illegible]nology (CST, Danvers, MA, USA) provided [illegible]rabbit anti-β-[illegible] and rabbit anti-STAT3 (signal [illegible]ransducers a[illegible]activators of transcription 3), a[illegible]l as goa[illegible]ti-rabbit secondary antibody. Che[illegible]t film was applied for assessment of protein expression with Image J software (NIH, Bethesda, MD, USA).

Cell Proliferation Assay

Cervical cancer cells were seeded in the 96-well plate (1×10^3 cells/well). After that, we added cell-counting kit-8 (CCK-8) (10 μL) (Dojindo, Kumamoto, Japan) into these wells at the appointed time points. Microplate reader was used for measuring absorbance at 450 nm (Bio-Rad, Hercules, CA, USA).

Wound Healing Assay

Cells seeded into 6-well plates were cultured in RPIM-1640 medium overnight. After scratched with a plastic tip, cells were cultured in serum-free RPMI-1640. Wound closure was viewed at the appointed time points. Each assay was independently repeated in triplicate.

Transwell Assay

5×10^4 cells in 200 μL serum-free RPMI-1640 were applied on the top side of the transwell chamber (8 μm in pore size, Millipore, Billerica, MA, USA) pre-coated with 50 μg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The bottom chamber was added with RPMI-1640 containing 10% fetal bovine serum (FBS). 48 h later, after wiped by cotton swab, the top surface of chambers was immersed for 10 min with precooling

methanol and stained in crystal violet for 30 min. Invasive cells were counted in three randomly selected fields per well (magnification 40×).

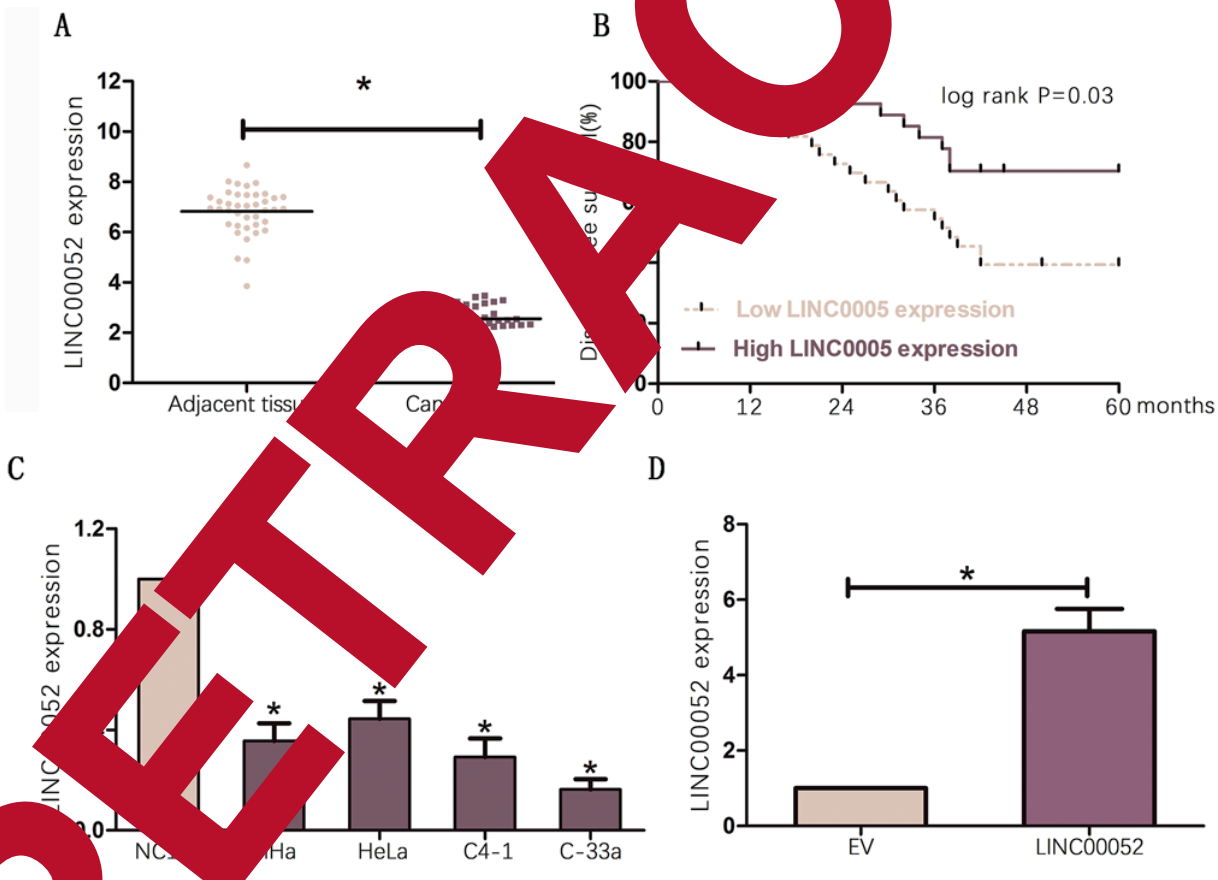
Statistical Analysis

Statistical analysis was conducted through Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA). Chi-square test, Student *t*-test and Kaplan-Meier method were utilized. Data were presented as mean ± SD (Standard Deviation). $p<0.05$ was considered of statistically significance.

Results

LINC00052 Expression Level in Cervical Cancer Tissues and Cells

First, qRT-PCR was conducted for detecting LINC00052 expression in 60 paired cervical cancer tissues and 4 cervical cancer cell lines. As a result, LINC00052 was si... downregulated in tumor tissue s... ...ures (Figure 1A). The overall survival of ...ical cancer patients after surgery was a...ed through Kaplan-Meier method. A tota... cervical cancer patients were ...ivided ...high LINC00052 and low-L...00052 grou... on the median expres... level ...LINC0... Kaplan-Meier anal... show... that cervical cancer patients w... the ...NC00... level had a better ...sea... ...surviv... (Figure 1B). LINC00... level w... ...yp... ...ated in cervical ca... cells than t... ...ormal cervical epit... ...ll line NC10... (Figure 1C). In our ...y, we ... C-33a cervical cancer cell line for the over... ...ssion of LINC00052. QR... was utilized ... detecting the trans-f... ...efficacy (Figure 1D).



Figu... Expression level of LINC00052 was downregulated in cervical cancer tissues and cell lines and associated with better ...val of cervical cancer patients. ***A,*** LINC00052 expression was significantly downregulated in the cervical ... tissues compared with adjacent tissues. ***B,*** High level of LINC00052 was associated with better disease-free survival of ...ical cancer patients. ***C,*** Expression level of LINC00052 relative to β-actin was determined in the human cervical cancer cell ...d normal cervical epithelium cell line NC104 by qRT-PCR. ***D,*** LINC00052 expression in cervical cancer cells transfected w... ...00052 lentiviruses (LINC00052) and the empty vector (EV) was detected by qRT-PCR. β-actin was used as an internal co... Data are presented as the mean ± standard error of the mean. *$p<0.05$.

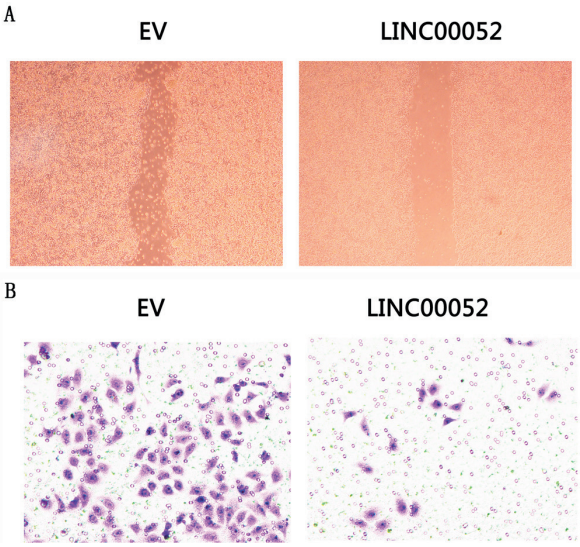


Figure 2. Overexpression of LINC00052 promoted cervical cancer cell migration and invasion. ***A,*** Wound healing assay showed that overexpression of LINC00052 significantly repressed cell migration in cervical cancer cells. ***B,*** Transwell assay showed that number of invaded cells significantly decreased *via* overexpression of LINC00052 in cervical cancer cells. The results represent the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

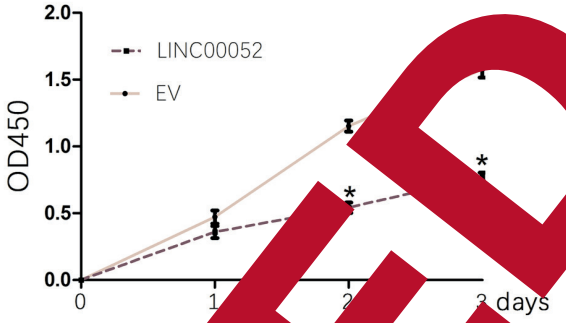


Figure 3. Overexp[illegible]on o[illegible]052 inhi[illegible] cervical cancer cell prolife[illegible]. CCK8 a[illegible]wer[illegible] cell viability significantly [illegible]eased *via* overe[illegible] LINC00052 in cervical ca[illegible] The results rep[illegible] the average of three inden[illegible]t e[illegible]nts (mean ± standard error of the mean). *p [illegible]5, as co[illegible]with the control cells.

Overexpression of LINC00052 Inhibi[illegible] Cell Migration and Invasion in Cervica[illegible] Cancer Cells

Results of wound healing [illegible]aled that the wound closure of cells si[illegible]canti[illegible]creased after overexpression of LI[illegible]0052 in [illegible]3a cells (Figure 2A). Transwell [illegible]also the invasive ability o[illegible]cells [illegible]arkably repressed through ove[illegible]ressio[illegible]NC00052 in C-33a cells (Figu[illegible]).

Overexpres[illegible]n o[illegible]00052 Inhibited Cell Grow[illegible]h in Cervic[illegible]ncer Cells

CCK-8 [illegible]y was conduct[illegible] detecting cell growth [illegible]ity. The results re[illegible]aled that after LINC[illegible]52 wa[illegible]erexpressed in C-33a cells, the [illegible]rowth [illegible]lity w[illegible]ignificantly repressed (Figu[illegible]).

[illegible]nterac[illegible]ween STAT3 and LIN[illegible]0052 in[illegible]rvical Cancer

Q[illegible]PCR results showed that compared with th[illegible] in empty vector (EV) group, [illegible]ession level of STAT3 in cervical can[illegible]cells was lower in LINC00052 lentiviruses ([illegible]00052) group (Figure 4A). Western blot foun[illegible]at overexpression of LINC00052 down-[illegible]ulated protein level of STAT3 in cervical [illegible]cer cells (Fig[illegible] 4B). We further found that [illegible]T3 expressio[illegible]f cervical cancer tissues was o[illegible]ly upre[illegible]ed compared with that of adjace[illegible]gure 4C). Correlation analysis demonstrated that STAT3 expression level was [illegible]gatively correlated to LINC00052 expression [illegible]issues (Figure 4D).

Discussion

LncRNAs have been reported to be associated with pathogenesis of many cancers. Recently, evidence proved that lncRNAs serve as a crucial part in the development of cervical cancer. For instance, by regulation of miR-21-5p, lncRNA MEG3 functions as a tumor-suppressor gene in cervical cancer to inhibit tumor growth[9]. LncRNA HOTAIR promotes the proliferation and invasion of cervical cancer cells through targeting the Notch pathway, which may be a potential treatment target[10]. LncRNA CCAT2 promotes cell proliferation and survival in cervical cancer[11]. Increased expression level of lncRNA CCHE1 is associated with poor prognosis of cervical cancer, which could be a potential prognostic marker[12]. In addition, overexpression of lncRNA NNT-AS1 facilitates the proliferation and invasion of cervical cancer cell *via* Wnt/beta-catenin signaling pathway[13]. Recently, lncRNA LINC00052 has been reported to participate in tumorigenesis of multiple cancers. For instance, overexpression of LINC00052 promotes the progression of breast cancer by HER3-me-

diated downstream signaling, which could be used as a potential diagnostic and therapeutic marker[14]. Through activating Wnt/beta-Catenin signaling pathway, lncRNA LINC00052 promotes the proliferation and metastasis of gastric cancer cells[15]. In addition, lncRNA LINC00052 inhibits migration and invasion of hepatocellular carcinoma cells through upregulating EPB41L3 *via* miR-452-5p[16]. Our current study demonstrated that LINC00052 was downregulated both in cervical cancer samples and cell lines. Besides, expression level of LINC00052 was closely related to the prognosis of cervical cancer. Furthermore, after LINC00052 was overexpressed, cervical cancer cell migration and invasion were found to be suppressed. Above results indicated that LINC00052 inhibited tumorigen vical cancer as a tumor suppress transducer and activator of trans tion (STAT) transcription factors have bee cated to be widely expressed in hematologi various cell types. It has been re ted tha ions such as STAT3, are a ated with immunodeficiency sy mes a tumori sis[17,18]. For example 6/JAK AT3 pathway functions as a cruc in develo nt of colorectal cance vhi contri to develop potentia rapeutic ach . STAT3 is activated re than 40% st cancers, which pr progression breast tumor

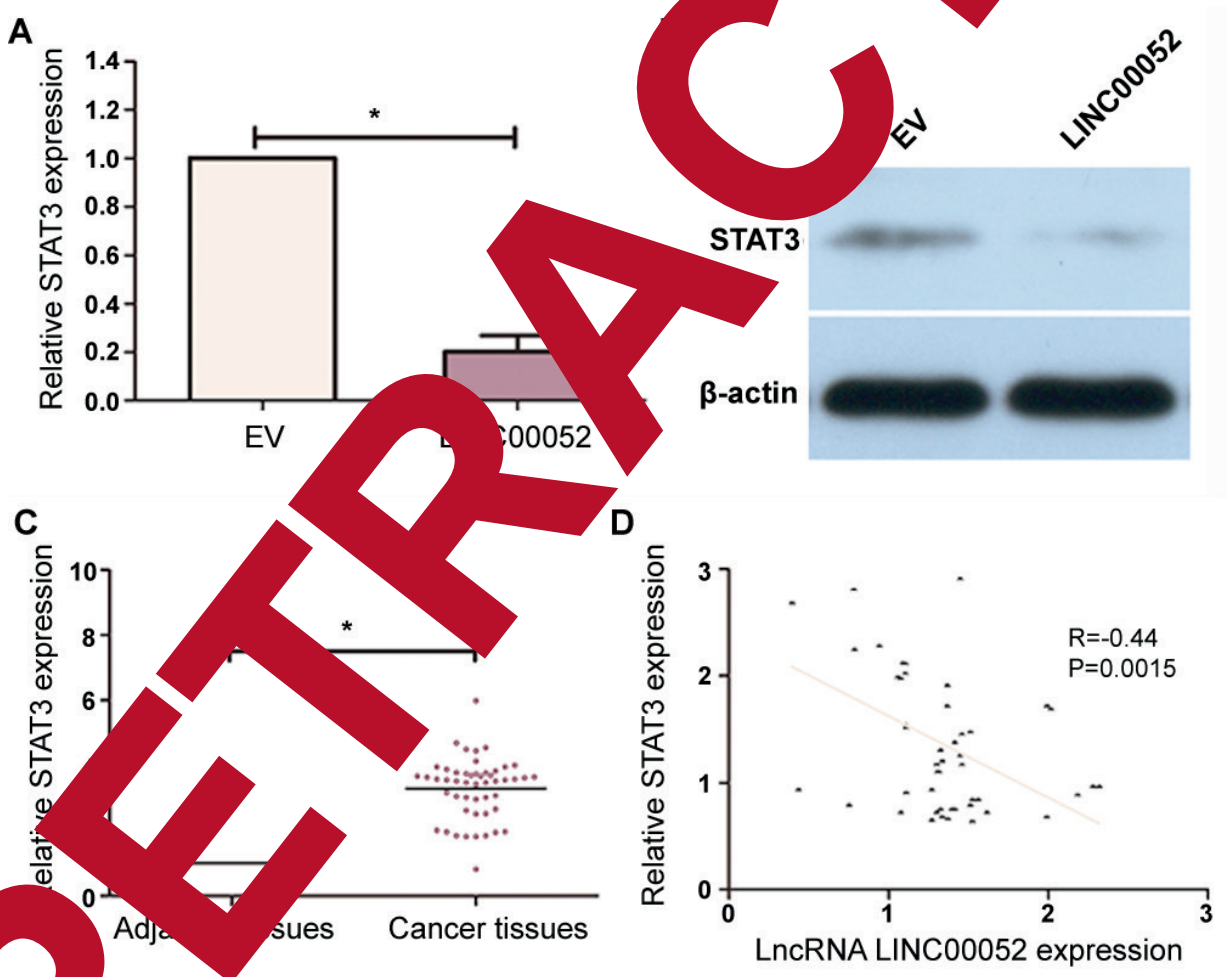


Fig n between LINC00052 and STAT3. ***A,*** QRT-PCR results showed that STAT3 expression was lower in 00052 len iruses (LINC00052) group compared with the empty vector (EV) group. ***B,*** Western blot assay revealed that T3 protein expression decreased in LINC00052 lentiviruses (LINC00052) group compared with the empty vector (EV) ***C,*** STAT3 was significantly upregulated in cervical cancer tissues compared with adjacent tissues. ***D,*** The linear correlati n the expression level of STAT3 and LINC00052 in cervical cancer tissues. The results represent the average of three indep nt experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

by regulating expressions of downstream target genes[20]. Overexpression of STAT3 promotes tumor progression and metastasis of ovarian cancer cells, highlighting a potential therapeutic target for ovarian cancer[21]. Moreover, inhibited by WP1066, the STAT3 signaling pathway depresses the growth and invasiveness of bladder cancer cells[22]. In the present study, mRNA and protein expressions of STAT3 were downregulated *via* overexpressing LINC00052. STAT3 expression was higher in cervical cancer tissues. Moreover, STAT3 expression in cervical cancer tissues was negatively related with LINC00052 expression. All the above results suggested that LINC00052 might repress tumorigenesis of cervical cancer *via* STAT3.

Conclusions

We identified that LINC00052 was remarkably downregulated and was correlated to disease-free survival of cervical cancer patients. Besides, LINC00052 could suppress cell growth, [illegible] tion and invasion of cervical cancer cell b[illegible] regulating STAT3. LINC00052 may serv[illegible] a therapeutic target for cervical cancer.

Conflict of Interests

The Authors declare that they ha[illegible] conflic[illegible] interests.

Ref[illegible]

1) Ferlay J, Soerjo[illegible]am I, Dikshit [illegible] Mathers C, Rebelo M[illegible]M, Forman D, [illegible]F. Cancer incide[illegible]ality worldwide: sources, methods [illegible]major p[illegible] in GLOBOCAN 2012. Int J Ca[illegible]er 2015; 136: [illegible]386.

2) Sieg[illegible]Naishadham D, Jemal[illegible]ancer statistics, 20[illegible]A Canc[illegible]r J Clin 2013; 63: 11-30.

3) [illegible]WJ, B[illegible]MA, Bajaj A, Chuang LT, Fisher BJ, [illegible]nride[illegible]i, Jhing[illegible]A, Kitchener HC, Mileshk[illegible]Nathan A[illegible]affney DK. Cervical cancer: [illegible]health[illegible]s. Cancer-Am Cancer Soc 017; 12[illegible]4.

4) [illegible]G, Tang[illegible]ang JF, Qi S, Yu GD, Sun C. Up[illegible]lation of l[illegible]g noncoding RNA CCAT2 indi[illegible] prognosis and promotes proliferation [illegible]sis in intrahepatic cholangiocarcinoma. Mol Med Rep 2018; 17: 5328-5335.

[illegible]iu G, Hu X, Zhou G. Long non-coding RNA OR3A4 [illegible]motes proliferation and migration in breast can[illegible]Biomed Pharmacother 2017; 96: 426-433.

6) Xiong Y, Wang L, Li Y, Chen M, He W, Qi L. The long non-coding RNA XIST interacted with [illegible]e modulate bladder cancer growth, migration by targeting androger[illegible]eptor (AR). Cell Physiol Biochem 2017; 43: [illegible]418.

7) Qi H, Wen B, Wu Q, Cheng W, [illegible]Yei J, Huang J, Yao X, Weng G. Long noncodi[illegible]SNHG7 accelerates prostate c[illegible]er pro[illegible] and cycle progression thr[illegible]cyclin D1 [illegible] ing miR-503. Biome[illegible]armac[illegible]ther 201[illegible] 326-332.

8) Vennin C, Spruyt [illegible]in YM[illegible]ssat T, [illegible]Bourhis X, Adriaenssens E. [illegible]-coding[illegible]A 91H increases a[illegible]sive[illegible]ype of[illegible]ast cancer cells a[illegible]p-regulat[illegible]/IG[illegible]xpression through [illegible]enetic modific[illegible]Cancer Lett 2017; [illegible]06.

9) Zha[illegible]hao [illegible]Y, Yu J, Liu Y, Lin Z. Long non-coding RNA ME[illegible]lownregulated in cervical c[illegible]and affects c[illegible]liferation and apoptosis [illegible]ulating miR-21. C[illegible]er Biol Ther 2016; 17: 104-113.

Lee M, Kim H[illegible] SW, Park SA, Chun KH, Cho NH, Song YS, Kim [illegible]The long non-coding RNA HOTAIR increas[illegible]umour growth and invasion in [illegible]vical can[illegible]by targeting the Notch pathway. [illegible]6; 7: 44558-44571.

11) Wu [illegible], Zhang W, Zhang L. Roles of long non-coding RNA CCAT2 in cervical cancer cell growth and [illegible]optosis. Med Sci Monit 2016; 22: 875-879.

[illegible], Wang CX, Sun XX, Wang C, Liu TF, Wang DJ. Long non-coding RNA CCHE1 overexpression predicts a poor prognosis for cervical cancer. Eur Rev Med Pharmacol Sci 2017; 21: 479-483.

13) Hua F, Liu S, Zhu L, Ma N, Jiang S, Yang J. Highly expressed long non-coding RNA NNT-AS1 promotes cell proliferation and invasion through Wnt/beta-catenin signaling pathway in cervical cancer. Biomed Pharmacother 2017; 92: 1128-1134.

14) Salameh A, Fan X, Choi BK, Zhang S, Zhang N, An Z. HER3 and LINC00052 interplay promotes tumor growth in breast cancer. Oncotarget 2017; 8: 6526-6539.

15) Shan Y, Ying R, Jia Z, Kong W, Wu Y, Zheng S, Jin H. LINC00052 promotes gastric cancer cell proliferation and metastasis via activating the wnt/beta-catenin signaling pathway. Oncol Res 2017; 25: 1589-1599.

16) Zhu L, Yang N, Chen J, Zeng T, Yan S, Liu Y, Yu G, Chen Q, Du G, Pan W, Li X, Zhou H, Huang A, Tang H. LINC00052 upregulates EPB41L3 to inhibit migration and invasion of hepatocellular carcinoma by binding miR-452-5p. Oncotarget 2017; 8: 63724-63737.

17) Uzel G, Sampaio EP, Lawrence MG, Hsu AP, Hackett M, Dorsey MJ, Noel RJ, Verbsky JW, Freeman AF, Janssen E, Bonilla FA, Pechacek J, Chandrasekaran P, Browne SK, Agharahimi A, Gharib AM, Mannurita SC, Yim JJ, Gambineri E, Torgerson T, Tran DQ, Milner JD, Holland SM. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune

dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. J Allergy Clin Immunol 2013; 131: 1611-1623.

18) Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, Freeman AF, Demidowich A, Davis J, Turner ML, Anderson VL, Darnell DN, Welch PA, Kuhns DB, Frucht DM, Malech HL, Gallin JI, Kobayashi SD, Whitney AR, Voyich JM, Musser JM, Woellner C, Schaffer AA, Puck JM, Grimbacher B. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 2007; 357: 1608-1619.

19) Wang SW, Sun YM. The IL-6/JAK/STAT3 pathway: potential therapeutic strategies in treating colorectal cancer (Review). Int J Oncol 2014; 44: 1032-1040.

20) Banerjee K, Resat H. Constitutive activation of STAT3 in breast cancer cells: a review[illegible] cer 2016; 138: 2570-2578.

21) Saini U, Naidu S, ElNaggar AC, [illegible]K, Wallbi[illegible] lich JJ, Bixel K, Bolyard C, S[illegible] AA, Kaur B, Kuppusamy P, Hays J, Goodfe[illegible] Cohn DE, Selvendiran K. Elevated STAT[illegible] ssion in ovarian cancer ascites [illegible]motes [illegible]n and metastasis: a potentia[illegible]rapeutic t[illegible] cogene 2017; 36: 16[illegible].

22) Tsujita Y, Horiguchi A[illegible]aki S, Is[illegible]M, Asano T, [illegible]o K, Asano T, Mayu[illegible]ushibiki[illegible]AT3 inhibition by WP1066 suppresse[illegible]and inva[illegible]ess of bladder cance[illegible]s. O[illegible] 2017; 3[illegible]7-2204.