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Long noncoding RNA LUCAT1 promotes cervical cancer cell proliferation and invasion by upregulating MTA1

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Abstract. – OBJECTIVE: Recent researches have revealed the role of long noncoding RNAs (lncRNAs) in the development of tumors. In this study, lncRNA LUCAT1 was explored to identify how it affected the progression of cervical cancer.

PATIENTS AND METHODS: Quantitativ al-time polymerase chain reaction (qR was used to detect LUCAT1 expression i th cervical cancer cells and tissue samples. M over, the associations between LUCAT1 exp sion level and patients' overall urvival ra were explored, respectively. ion, ce proliferation assay and tra ell y were conducted. Furthermore, t nderly mechanism was explored via p ming q and Western blot assay.

RESULTS: By co ing w e expression level in corresp g ones, UCAT1 expression level i ical cancer les was significantly l er. over, expre ion level of LUCAT1 w negat correlated with patients' ov survival t In addition, after LUCAT1 s overexpresse ll proliferation, cell i sion a migration capacities were pro ed *in* . In addition, the mRNA and pro xp ions of TA1 were upregulated after L was o pressed. Furthermore, s fo hat expression level of MTA1 w ositiv ed to LUCAT1 expression lev cervic ncer tissues.

CLUSIONS: We showed that LUCAT1 e proliferation, invasion and mi- tion of cervical cancer cells through upreg- ting MTA1, which might offer a potential ther- tic choice for patients with cervical cancer.

Key Words:
Long noncoding RNA, LUCAT1, MTA1, Cervical cancer.

Introduction

Cervical can is the fourth most common n ancy in f le all over the world and the mos ale ncer among Chinese women. IARC p s indicated that more than half a million cases were newly diagnosed as breast in 2012, which accounts for 7.5% of fe- ncer-related deaths[1]. Basic treatments for patients with cervical cancer include surgery or a concurrent chemoradiotherapy, which consists of cisplatin-based chemotherapy and radiotherapy. The prognosis of patients with metastatic cervical cancer is very poor. In many developing countries, due to the grossly deficient treatments, cervical cancer kills more than 250,000 women annually[2]. Therefore, it is urgent to find out the underlying mechanism and figure out a new treatment strategy for these unfortunate women. Most of genome transcripts are non-coding RNAs. Long non-coding RNAs (lncRNAs) are one subtype of non-coding RNAs, which are longer than 200 nucleotides in length. In recent years, lncRNAs are widely studied in a variety of biological behaviors. Moreover, evidences have proved that lncRNAs act as a vital role in the progression of malignant tumors. For example, LncRNA PlncRNA-1 acts as an oncogene in the progression of colorectal cancer cell through regulating PI3K/Akt Signaling Pathway[3]. LncRNA SNHG1 could inhibit the differentiation of Treg cells, thereby impeding the immune escape of breast cancer[4]. LncRNA p23154 accelerates metastasis[5] in oral squamous cell carcinoma by taking part in glycolysis mediated by Glut1. Moreover, overexpression

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of lncRNA CCAT2 has been proved to promote the proliferation and metastasis in intrahepatic cholangiocarcinoma[6]. However, the role of lncRNA LUCAT1 plays in cervical cancer and the underlying molecular mechanism of how it works remains unexplored. In our study, we found out that LUCAT1 expression level was remarkably higher in cervical cancer tissues. Moreover, LUCAT1 promoted the proliferation, invasion and migration capacities of cervical cancer cell *in vitro*. In addition, our further experiment explored the underlying mechanism of how LUCAT1 functioned in the development of cervical cancer.

Patients and Methods

Cell Lines and Clinical Samples

A total of 62 cases that were diagnosed as cervical cancer patients were enrolled in this research. Every patient received surgery at Affiliated Hospital of Yan'an University. Before the surgery, all the written informed consents were gathered. No radiotherapy or chemotherapy for any patients before the operation. Tissues were collected from the surgery and stored immediately at -80°C. All tissues were analyzed by two experienced pathologists. The Research Ethics Committee of Affiliated Hospital of Yan'an University granted the approval for the study.

Cell Culture

SiHa, HeLa, Caski, C and C cervical cancer cell lines, a normal epithelial cell (NC104) and 2 cell (C Type Culture Collection, ese Academy Sciences, Shanghai, Chi used in this dy. Culture medium was consisted of penicillin, Dulbecco's Modified Eagle Medium (DMEM, HyClone, South Logan, UT, USA) and 10% fetal bovine serum (FBS; G , Rockville, MD, USA). Besides, cells were cultured in humidified incubator, which contained 5% C and was set at 37°C.

Trans

er synthesized, lentiviral virus targeting LU was cloned into the pLenti-EF1a-EGFP-EF1 tor (Biosettia Inc., San Diego, CA, USA). LUCAT1 lentiviruses (LUCAT1) and the empty vector (control) packaged in 293T cells were then used for transfection in cervical cancer cells. 48 h later, detection of LUCAT1 expression level in these cells was conducted using quantitative Real-time polymerase chain reaction (qRT-PCR).

RNA Extraction and qRT-PCR

The total RNA was separated by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). And then, the total RNA was reverse-transcribed to complementary deoxyribose nucleic acids (cDNAs) through reverse Transcription (TaKaRa Biotechnology Co., Ltd., Dalian, C Following are the primers using for RT-qPCR. AT1, forwards 5′-CCTATCCCTTTC TAAG and reverse 5′-ACTTCTGC AACGTC MTA1, forward 5′-TA GACT AGAT GAGATGGCCGCC ATGT G-3′ and reverse 5′-GATCC CGGT GGT-GTCCTCGATG ATC TC-3′; APDH, forward 5′ AAATC GCAAT-GCTGG-3′ everse 5′ GGCATG-GACTGT TCA A-3′. The thermal cycle was as follows: 30 s °C, 5 s at 95°C for 40 cycles at 60°C.

Western Blot Analysis

eagent ra mmunoprecipitation assay ( ) (Beyoti Shanghai, China) was utilized tra tein from cells. Bicinchoninic acid (tein assay kit (TaKaRa, Dalian, China) was chosen for quantifying protein concentrations. The target proteins were separated m dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, they were incubated with antibodies after replaced to the polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland). Cell Signaling Technology (CST, Danvers, MA, USA) provided us with rabbit anti-GAPDH and rabbit anti-MTA1, as well as goat anti-rabbit secondary antibody. Chemiluminescent film was applied for assessment of protein expression with Image J software.

Colony Formation Assay

After cultured with fetal bovine serum (FBS) in a 6-well plate for 14 days, all cells were fixed with methanol and stained with 0.1% crystal violet. Meanwhile, we counted number of colonies for comparison.

Cell Counting Kit-8 (CCK8) Assay

Cell growth of theses treated cells in 96-well plates was monitored every 24 h by CCK8 assay by following the protocol (Dojindo Molecular Technologies, Inc.). The absorbance was examined at 450 nm *via* Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

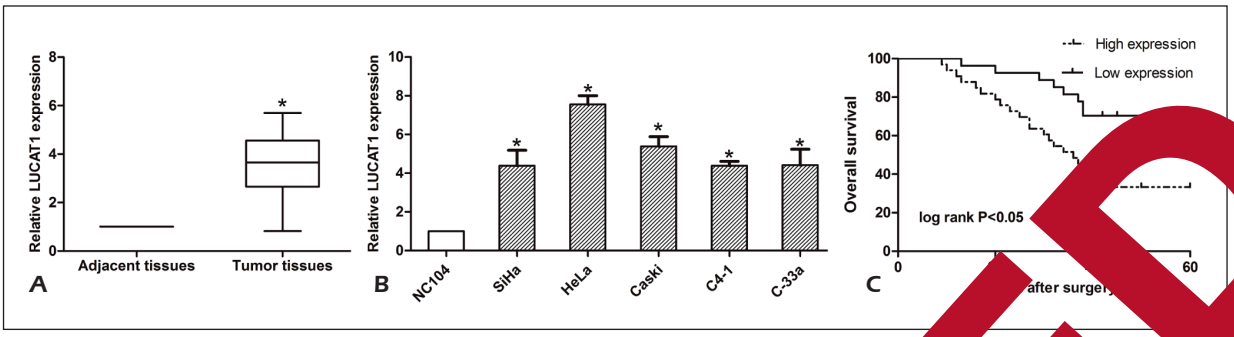

Figure 1. Expression levels of LUCAT1 were increased in cervical cancer tissues and cell l[illegible] which [illegible] associated [illegible] poorer overall survival of cervical cancer patients. ***A***, LUCAT1 expression was significantly i[illegible]d in [illegible]ervical c[illegible]cer tissues compared with adjacent tissues. ***B***, Expression levels of LUCAT1 relative to GAPDH [illegible]de[illegible] the hum[illegible]rvical cancer cell lines and NC104 (normal cervical epithelium cell line) by qRT-PCR. ***C***, Hi[illegible]vel of L[illegible] was a[illegible]ated with poorer overall survival of cervical cancer patients. Data are presented as the mean ± [illegible]ard error of t[illegible].05.

Matrigel Assay

5 ×10^4 cells in 200 µL of serum-free DMEM were transformed to top chamber of an 8 µm pore size insert (Millipore, Billerica, MA, USA) coated with or without 50 µg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). And the bottom chamber was added with Dulbecco's Mo[illegible]d Eagle's Medium (DMEM) and fetal bovin[illegible] (FBS). 48 h later, after wiped by cotton swa[illegible]e top surface of chambers was immersed for 10[illegible] with precooling methanol and stained in crys[illegible] violet for 30 min. Three fields w[illegible]d to cou[illegible] the data for invasion membra[illegible]

Statistical Analysis

Statistical Produc[illegible]and [illegible]Sol[illegible]s (SPSS) 20.0 (IBM [illegible]honk, [illegible]SA) was utilized to conduct [illegible]stical analy[illegible]Data were presented as [illegible]. Student *t*[illegible], x^2-test and Kaplan-M[illegible]er met[illegible]ere performed when appropria[illegible]When $p<0.0$[illegible]as considered statistical[illegible]nificant.

R[illegible]lts

[illegible]ssio[illegible]of LUCAT1 in [illegible]vical c[illegible]er Tissues and Cells

[illegible] the expression of LUCAT1 was de[illegible]d [illegible]forming qRT-PCR in 62 patients' [illegible]mples and 4 cervical cancer cell lines. The [illegible]lts revealed that LUCAT1 was significantly up[illegible]dated in tumor tissue samples (Figure 1A). Expression level of LUCAT1 in cervical cancer cells was remarkably higher than that of NC104 (normal cervical epithelium cell line) (Figure 1B).

LUC[illegible]xpression [illegible]Correlated W[illegible]Overall Survival [illegible]me of Cervical [illegible]cer Patient[illegible]

[illegible]aplan-Meie[illegible]ethod was utilized to analyze p[illegible]ts' surviva[illegible]me after the surgery. By media[illegible]essio[illegible] cervical cancer patients were randomi[illegible]ed into two groups, the low-LUCAT1 group and high-LUCAT1 group. The results [illegible]lan-Meier analysis showed that cervical [illegible]atients had a better overall survival with lower LUCAT1 level (Figure 1C).

Overexpression of LUCAT1 Promoted Cell Proliferation in Cervical Cancer

HeLa cervical cancer cell line was used for the overexpression of LUCAT1 in this study. The LUCAT1 expression was detected by qRT-PCR (Figure 2A). Then outcome of colony formation assay revealed that overexpression of LUCAT1 promoted ovarian cancer cell growth (Figure 2B). Moreover, results of cell proliferation assay indicated that the growth ability of cervical cancer cells was significantly facilitated after LUCAT1 was overexpressed (Figure 2C).

Overexpression of LUCAT1 Enhanced Migration and Invasion in Cervical Cancer Cells

The outcome of wound healing assay revealed that after LUCAT1 was overexpressed, the migration ability of cervical cancer cells was promoted (Figure 3A). Furthermore, the results of transwell assay also revealed that the quantity of migrant and invaded cells was remarkably increased after LUCAT1 was overexpressed in cervical cancer cells (Figure 3B and 3C).

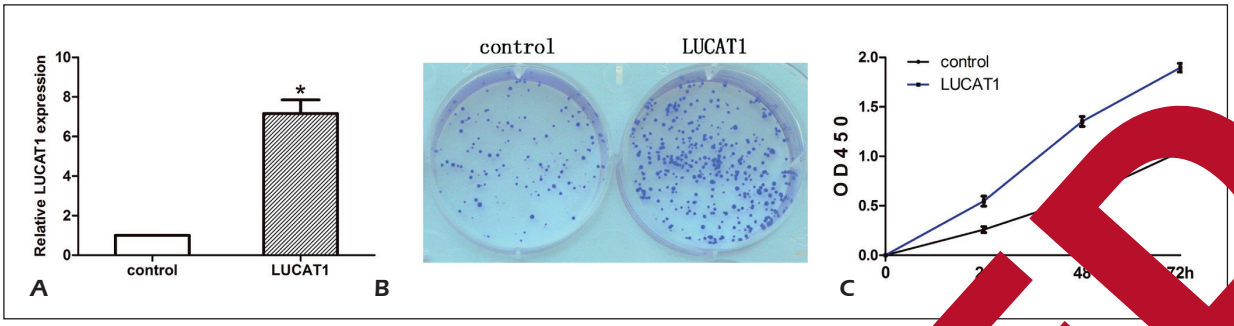

Figure 2. Overexpression of LUCAT1 promoted cervical cancer cell proliferation. ***A***, LUCA[T1] expressio[n] [in] cervical ca[ncer] cells transduced with LUCAT1 lentiviruses (LUCAT1) and the empty vector (control) w[ere] detected b[y] [q]RT-PCR. GAPDH was used as an internal control. ***B***, Colony formation assay showed that number of colo[nies] [in] [LUCAT1] lentivirus[es] [gr]oup was significantly increased compared with empty control group in cervical cancer cells. [Cell pro]liferation assay [al]so showed that overexpression of LUCAT1 significantly increased cell growth in cervical cancer [cells]. The result[s were] [rep]resented as average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as [compare]d with the co[ntrol cells].

The Interaction Between MTA1 and LUCAT1 in Cervical Cancer

The results of qRT-PCR showed that the expression level of MTA1 was significantly higher in LUCAT1 lentiviruses (LUCAT1) group when compared with that in empty vector (control) group (Figure 4A). The outcome of Western blot assay revealed that after LUCAT1 was overexpr[essed], MTA1 could be upregulated at protein lev[el] (Figure 4B). Furthermore, we found that MTA1 e[xpres]sion of cervical cancer tissues was significa[ntly] higher when compared with that of adjacent tiss[ues] (Figure 4C). Correlation analy[sis] demonstrate[d] that MTA1 expression level po[sitively] related to LUCAT1 expression in can[cer] tissues ([Fig]ure 4D).

Discussion

LncRNAs [have] proved to b[e] associated with pathogen[esis] of [many] cancers. Evidence revealed th[at] lncRNAs fu[nction] as a crucial part in the development o[f cervi]cal cancer. For example, [the] expression of [lncRNA] ZNF667-AS1 re[gulat]es the progression of cervical cancer, which [is] also related to [the] prognosis of cervical cancer[7]. [Up]-expression [of] lncRNA PANDAR promotes th[e gr]owth of c[ervi]cal cancer cells and predicts the [poor] prognosis of patients with cervical cancer[8]. [Lo]ng lncRNDE enhances the proliferation and metastasis of cervical cancer cells[9]. Through [re]gulation of miR-21-5p, lncRNA MEG3 acts [as] a [tu]mor suppressor in cervical cancer, leading to the inhibition of tumor growth[10].

Lung cancer associated transcript 1 (LUCAT1) is a long noncoding RNA located on chromosome 5, which was found in the airway epithelium of cigarette smokers firstly[11]. Recently, lncRNA is widely explored for its important role in the development of tumors. For instance, overexpression of LncRNA LUCAT1 could promote cell proliferation in human non-small lung cancer through inhibiting the expression of p21 and p57[12]. LncRNA LUCAT1 inhibits

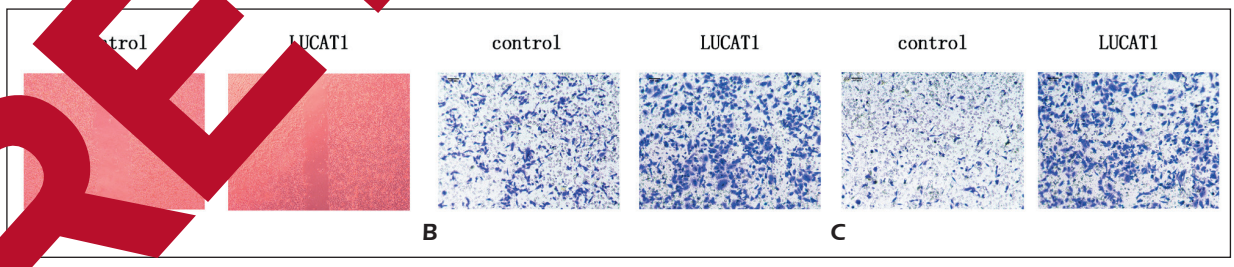

Figure 3. Overexpression of LUCAT1 promoted cervical cancer cell migration and invasion. ***A***, Wound-healing assay showed [that] migrated length of cervical cancer cells was significantly increased via overexpression of LUCAT1 in cervical cancer cells. ***B***, Transwell assay showed that number of migrating cells was significantly increased via overexpression of LUCAT1 in cervical cancer cells (magnification, 40×). ***C***, Transwell assay showed that number of invading cells was significantly increased via overexpression of LUCAT1 in cervical cancer cells (magnification, 40×). The results represent the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

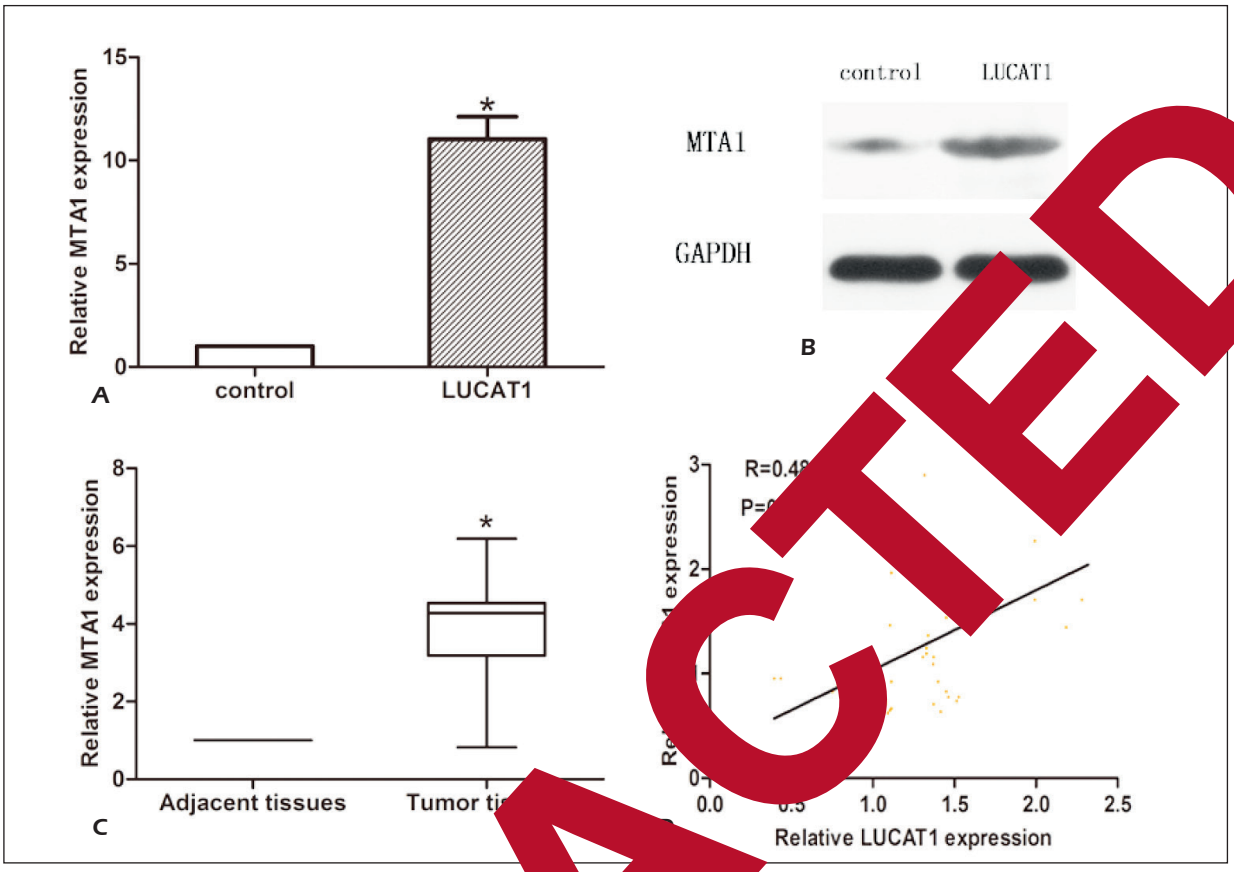

Figure 4. Interaction between LUCAT1 and MTA1. A, MTA1 result showed that MTA1 expression was higher in LUCAT1 lentiviruses (LUCAT1) compared with the empty (control). *B*, Western blot assay revealed that MTA1 protein expression was increased in LUCAT1 (LUCA mpared with the empty vector (control). *C*, MTA1 was significantly upregulated in cervical canc ared with ent tissues. *D*, The linear correlation between the expression level of MTA1 and LUCAT1 in al canc ssues. The lts represent the average of three independent experiments. Data are presented as the mea ndard er the mean. $p<0.05$.

the expression o or suppres nd leads to the forma vasion of phageal squamous cel arcino regulating the stability of T1[13]. In ad upregulation of lncRN CAT1 is signifi related to malign tage o ar cell renal cell carcinoma an dicts poor prognosis of patients with ccRC s rese we figured out that the ressi LUC was remarkably upregu- n bo cancer samples and cells. Be s, the c elationship was observed between patients' prognosis and expression level LUC Furthermore, after LUCAT1 was overexpressed, the proliferation, invasion and ration of cervical cancer cell were promoted. The results above indicated that LUCAT1 promoted tumorigenesis of cervical cancer and might act as an oncogene. Metastasis-associated protein 1 (MTA1), as an essential component of the deacetylase (NuRD) complex and nucleosome remodeling, has been revealed to be a new factor that promotes the progression of epithelial-to-mesenchymal transition (EMT)[15]. For instance, through AKT/GSK3β/β-catenin signaling, MTA1 promotes cell EMT and metastasis in non-small-cell lung cancer[16]. Increased levels of MTA1 promote cell invasion and metastasis in human hepatocellular carcinoma by regulating ErbB2[17]. Through repression of MTA1 expression, miR-183 could inhibit the EMT and progression of human pancreatic cancer[18]. In the present study, MTA1 expression could be upregulated after overexpression of LUCAT1. Moreover, MTA1 expression in cervical cancer samples was positively related to LUCAT1 expression. All the results above suggest that LUCAT1 might promote tumorigenesis of cervical cancer *via* upregulating MTA1.

Conclusions

We showed that LUCAT1 was remarkably upregulated and was negatively related to overall survival time of patients with cervical cancer. Besides, LUCAT1 could enhance cell proliferation, invasion and migration in cervical cancer cells through upregulating MTA1. These findings suggest that LUCAT1 may contribute to therapy for cervical cancer as a candidate target.

Conflict of Interests

The authors declared no conflict of interest.

References

1) Di J, Rutherford S, Chu C. Review of the cervical cancer burden and population-based cervical cancer screening in China. Asian Pac J Cancer Prev 2015; 16: 7401-7407.
2) Small WJ, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, Jhingran A, Kitchener HC, Mileshkin LR, Viswanathan AN, Gaffney DK. Cervical cancer: a global health crisis. Cancer 2017; 2404-2412.
3) Song W, Mei JZ, Zhang M. Long noncodi NA PlncRNA-1 promotes colorectal cancer ce gression by regulating the PI3K/Akt sign pathway. Oncol Res 2018; 26: 261-268.
4) Pei X, Wang X, Li H. LncRNA regulate the differentiation of Treg fects the immune escape of brea ncer v egulating miR-448/IDO. Int J Bio romol 2 118: 24-30.
5) Wang Y, Zhang X, Z, J, Li X, Chen X, Wu T, Tao X, X, Li X, Cheng B. LncRNA-p23154 otes the inv metastasis potential of amous cell oma by regulating 1-m d glycolysis. ancer Lett 2018; 4 72-183.
6) Bai J ng RF, Shang S, Yu GD, Sun C. Up ation of long nonco RNA CCAT2 indi- a poor gnosis and promotes proliferation metas in intrahepatic cholangiocarcino- Rep 2 17: 5328-5335.
7) Zh RH, H , Zhang XQ, Nian GX, Wu MX, Zh , Sun ZG. Independent prog- stic F w-expressed LncRNA ZNF667-1 for ce cancer and inhibitory function on proliferation of cervical cancer. Eur Rev Med Sci 2017; 21: 5353-5360.
8) Huang HW, Xie H, Ma X, Zhao F, Gao Y. Upregulation of LncRNA PANDAR predicts poor prognosis and promotes cell proliferation in cervical cancer. Eur Rev Med Pharmacol Sci 2017; 21: 4529-4535.
9) Meng Y, Li Q, Li L, Ma R. The long ing RNA CRNDE promotes cervical growth and metastasis. Biol Che 017; 399 93-100.
10) Zhang J, Yao T, Wang Y, Yu J, L n Z. Long noncoding RNA MEG3 is downreg in cervical cancer and affects prolif and apoptosis by regulating 21. Cancer L 2016; 17: 104-113.
11) Thai P, Statt S, Chen Liang E pbell C, Wu X. Characterization of el l oncodi RNA, SCAL1, induce cig moke an vated in lung canc lines. Respir Mol Biol 2013; 49: 11.
12) Sun Y, J Q, Han L, Lu XY, Wang W, W , G Long non-c ing RNA LUCAT1 is ssociate poor prognosis in human n ell lung car d regulates cell proliferation epigenetical ressing p21 and p57 xpression. Oncotarget 017; 8: 28297-28311.
13) Yoon JH, You H, Park CH, Kim YJ, Nam JW, Lee SK. The long coding RNA LUCAT1 promotes umorigenesi controlling ubiquitination and bility of D methyltransferase 1 in esophageal cell carcinoma. Cancer Lett 2018; 417
14) Zheng Z, Zhao F, Zhu D, Han J, Chen H, Cai Y, Chen Z, Xie W. Long non-coding RNA LUCAT1 otes proliferation and invasion in clear cell renal cell carcinoma through AKT/GSK-3beta signaling pathway. Cell Physiol Biochem 2018; 48: 891-904.
15) Dhar S, Kumar A, Zhang L, Rimando AM, Lage JM, Lewin JR, Atfi A, Zhang X, Levenson AS. Dietary pterostilbene is a novel MTA1-targeted chemopreventive and therapeutic agent in prostate cancer. Oncotarget 2016; 7: 18469-18484.
16) Ma K, Fan Y, Dong X, Dong D, Guo Y, Wei X, Ning J, Geng Q, Wang C, Hu Y, Li M, Niu W, Li E, Wu Y. MTA1 promotes epithelial to mesenchymal transition and metastasis in non-small-cell lung cancer. Oncotarget 2017; 8: 38825-38840.
17) Deng L, Tang J, Yang H, Cheng C, Lu S, Jiang R, Sun B. MTA1 modulated by miR-30e contributes to epithelial-to-mesenchymal transition in hepatocellular carcinoma through an ErbB2-dependent pathway. Oncogene 2017; 36: 3976-3985.
18) Lin X, Zheng L, Song H, Xiao J, Pan B, Chen H, Jin X, Yu H. Effects of microRNA-183 on epithelial-mesenchymal transition, proliferation, migration, invasion and apoptosis in human pancreatic cancer SW1900 cells by targeting MTA1. Exp Mol Pathol 2017; 102: 522-532.