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LncRNA LINC00342 regulated cell growth and metastasis in non-small cell lung cancer *via* targeting miR-203a-3p

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Abstract. – OBJECTIVE: Non-small cell lung cancer (NSCLC) is the main form of lung cancer, leading to major causes of cancer mortality. It is well known that lncRNAs may be involved in the pathogenesis of cancer, including NSCLC. The aim of this study was to provide a novel therapeutic target of LINC00342 for the therapy of NSCLC.

PATIENTS AND METHODS: The expression of LINC00342 and miR-203a-3p was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Cell proliferation was measured using the MTT assay. Colony formation analysis was performed to count the number of colonies. Cell migration and invasion were measured by transwell. Online software DIANA tools were used to predict binding sites of LINC00342 and miR-203a-3p. Luciferase reporter assay was conducted to confirm the interaction between LINC00342 and miR-203a-3p.

RESULTS: The expression of LINC00342 was increased in NSCLC tissues and cells compared with normal tissues and cells. Knockdown of LINC00342 suppressed cell proliferation, colony formation, migration, and invasion. LINC00342 regulated the expression of miR-203a-3p by targeting it directly. MiR-203a-3p was down-regulated in NSCLC tissues and cells compared with normal tissues and cells. Furthermore, LINC00342 promoted NSCLC cells proliferation, colony formation, migration, and invasion by depleting the expression of miR-203a-3p.

CONCLUSIONS: This work implied that LINC00342 functions in NSCLC acting as an oncogene. Briefly, LINC00342 contributes to NSCLC cells growth and metastasis via targeting miR-203a-3p competitively.

Key Words:
Non-small cell lung cancer (NSCLC), LINC00342, MiR-203a-3p, Cell Growth, Metastasis.

Abbreviations

NSCLC = Non-small cell lung cancer; qRT-PCR = quantitative Real Time-Polymerase Chain Reaction; LncRNAs = Long non-coding RNAs, NcRNAs = non-coding RNAs; CDK4 = cyclin-dependent kinase 4; EMT = epithelial-mesenchymal transition; ADAM10 = A Disintegrin And Metalloproteinases 10; SiRNA = Small interfering RNA; si-LINC00342 = against LINC00342; LINC00342 = LINC00342 overexpression plasmid; anti-miR-203a-3p = antibodies against miR-203a-3p; DMSO = dimethylsulfoxide; OD values = absorbance values at 490 nm; PFA = paraformaldehyde; ANOVA = analysis of variance.

Introduction

Lung cancer is a major cancer which causes death worldwide. Non-small cell lung cancer (NSCLC) is the main form of lung cancer, accounting for more than 80% of all lung cancers[1]. In patients with NSCLC, approximately 65% have locally advanced or metastatic disease. Recently, despite the rapid progress of clinical medicine and oncology, especially in molecularly targeted drugs and immunological checkpoint inhibitors[3], the limited effect has made

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lung cancer prognosis worse, and the overall survival rate of patients is still unsatisfactory[4]. Therefore, there is an urgent need to understand the underlying pathological mechanisms of the development and progression of NSCLC, as well as to develop novel strategies for the treatment of NSCLC. Long non-coding RNAs (lncRNAs) are non-coding RNAs (ncRNAs) over 200 nucleotides in length with limited or no protein-coding capacity[5]. Recently, there is increasing evidence suggesting that lncRNAs may be involved in the pathogenesis of cancer, including NSCLC, providing distinct insights and references in the biology of this disease. For example, lncRNA PVT1 was significantly upregulated in NSCLC tissues, while the cell lines and knockdown of PVT1 markedly suppressed cell proliferation, migration, and invasion[6]. LncRNA HMlincRNA717 was downregulated in NSCLC, which was associated with poor overall survival of patients and the progression of tumor, suggesting its independent prognostic role in NSCLC[7]. LncRNA UCA1 expression was enhanced in NSCLC tissues, and its silencing inhibited proliferation and colony formation of NSCLC cells, suggesting UCA1 exerting oncogenic functions in NSCLC[8]. LINC00342 was regarded as one of lncRNAs, and its investigation was extremely limited. Thus, exploring LINC00342 in NSCLC was currently an attractive and promising project.

miRNAs are well known as a class of highly conserved non-coding RNAs comprising 18-23 nucleotides. Typically, miRNAs bind to the 3'-untranslated region of the mRNA (3'UTR), resulting in mRNA degradation and translational inhibition, which regulate expression of the target gene[9]. In recent years, numerous researches[10] claim that the dysregulation of miRNAs might disrupt the balance of cell signaling transduction and development processes, resulting in various diseases, including cancer. In NSCLC, several biological functions and regulatory mechanisms of miRNAs have been identified. For instance, miR-34b-3p suppressed cell proliferation and cell cycle by binding to the cyclin-dependent kinase 4 (CDK4) mRNA 3′-untranslated region (3′-UTR) in NSCLC[11]. The expression of miR-224 in NSCLC tumor tissues was significantly increased, and miR-224 mimic could enhance cell proliferation, reduced apoptosis, and autophagy in transfected starved A549 cells[12]. MiR-449a could delay the progression of epithelial-mesenchymal transition (EMT) and deplete invasion and migration by targeting A Disintegrin and Metalloproteinases 10 (ADAM10) in NSCLC[13]. Actually, miR-203a-3p acting as a tumor suppressor has been widely reported; however, its function in the development and metastasis of tumor in NSCLC has rarely been mentioned. In this work, for the first time, we measured the expression of LINC00342 and miR-203a-3p in NSCLC, and the interaction of LINC00342 and miR-203a-3p was observed. This research expands our understanding of the mechanisms of LINC00342 functioning on the tumor development and progression in NSCLC and may provide a novel therapeutic target for the treatment of NSCLC.

Patients and Methods

Samples and Cell Lines

A total of 75 paired NSCLC tissues and adjacent normal tissues were obtained from patients with NSCLC from the First Affiliated Hospital of Guangxi Medical University. All tissues were frozen in liquid nitrogen immediately after removal and stored at –80°C until use. This research was approved by the First Affiliated Hospital of Guangxi Medical University. All patients signed informed consent prior to clinical surgery.

Human lung cancer cell lines including NCI-H1299, NCI-H460, and A549. Human bronchial epithelial cell BEAS-2B were all purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). SPC-A-1 was obtained from BeNa Culture Collection (BNCC; Suzhou, China). All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA) at 37°C humidified condition containing 5% CO_2.

Cell Transfection

Small interfering RNA (siRNA) against LINC00342 (si-LINC00342), LINC00342 overexpression plasmid (LINC00342), the mature hsa-miR-203a-3p mimic, antibodies against miR-203a-3p (anti-miR-203a-3p), and the corresponding negative control were all purchased from GenePharma (Shanghai, China). Transfection of either miRNAs or siRNAs was conducted by Lipofectamine 2000 regent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Cells were harvested at 48 h after transfection for the following investigation.

RNA Out and QRT-PCR Analysis

Total RNA from tissues or cells was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the instruction of the manufacturer. Each RNA sample was reverse transcribed into cDNA using PrimeScript RT reagent kit (TaKaRa, Dalian, China). The RNA expression was detected by SYBR Premix Ex Taq II Kit (TaKaRa, Dalian, China) on the ABI 7900 system (Applied Biosystems; Foster City, CA, USA). The relative expression levels of LINC00342 and miR-203a-3p were normalized by GAPDH and U6, respectively. The relative expression levels were quantified by using the $2^{-\Delta\Delta Ct}$ method. All reactions were run in triplicate. The primers used were as follows: LINC00342, 5'-CCCAAAGCAGTCCTTCACTACA-3' (forward) and 5'-CTGCAGTTCACTCTGCTGCTT-3' (reverse); GAPDH, 5'-GACTCCACTCACGGCAAATTCA-3' (forward) and 5'-TCGCTCCTGGAAGATGGTGAT-3' (reverse); U6, 5'-CTCGCTTCGGCAGCACATATA-3' (forward) and 5'-AAATATGGAACGCTTCACGA-3' (reverse). The Bulge-Loop miRNA Primers for miR-203a-3p were provided by RiboBio Co., Ltd (Guangzhou, China).

MTT Assay

Transfected NCI-H1299 and NCI-H460 cells (4×10^3) were seeded into 96-well plates for 48 h. After incubation, 10 μl 3-(4, 5-dimethyldiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) was added into each plate and incubated for continuing 2 h. The supernatant was removed and 100 ml dimethylsulfoxide (DMSO) was added to dissolve formazan products. Finally, the absorbance values at 490 nm (OD values) were measured at 24, 48, 72, and 96 h by a microplate reader (Bio-Rad, Hercules, CA, USA).

Colony Formation

Transfected NSCLC cells (NCI-H1299 and NCI-H460) (4×10^3) were cultured in 6-well plates (Corning, Corning, NY, USA) in Dulbecco's Modified Eagle's Medium (DMEM) medium with 10% fetal bovine serum (FBS) at 37°C for 2 weeks. Later, cells were fixed and stained with 0.1% crystal violet for 20 min. Then, the number of colonies was observed under a microscope and counted using the ImageJ software.

Cell Migration and Invasion Analysis

Transfected NCI-H1299 and NCI-H460 cells (5×10^4) were harvested after culturing 48 h. After that, cells were re-suspended in serum-free Dulbecco's Modified Eagle's Medium (DMEM) and placed into the upper 24-well with 8 mm pore size transwell chamber (Corning, Costar , CA, USA) for migration assays or into the upper chamber well coated with Matrigel (Corning, Costar, CA, USA) for invasion assays. Meantime, Dulbecco's Modified Eagle's Medium (DMEM) solution supplemented with 10% fetal bovine serum (FBS) was added into the lower chamber. After an incubation at 37°C with 5% CO_2 for certain time, the migrated or invaded cells moved to the bottom surface and were fixed with 4% paraformaldehyde (PFA), and stained with methanol containing 0.1% crystal violet for 30 min. At last, five randomly selected fields were used to image and count cells number using an Olympus microscope (Tokyo, Japan).

Binding Sites Prediction and Luciferase Reporter Assay

The putative binding sites between LINC00342 and miR-203a-3p were predicted by online software DIANA-LncBase v2 (http://www.microrna.gr/LncBase).

Cells were seeded in the 24-well plates for 24 h. Then, LINC00342 wild-type (LINC00342-wt) or LINC00342 mutant (LINC00342-mut) sequences containing miR-203a-3p binding sites were constructed onto the downstream of pmirGLO expression vector (Promega, Madison, WI, USA) luciferase gene. After, LINC00342-wt or LINC00342-mut pmirGLO vectors and miR-203a-3p mimics, miR-NC, anti-miR-203a-3p or anti-miR-NC were con-transfected into osteosarcoma cell lines, respectively. Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) was used to detected the luciferase activities of NCI-H1299 and NCI-H460 cells at 48 h after transfecting.

Statistical Analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used to process the data. Each work was repeated at least three times and all data were presented as the mean ± SD from at least three independent investigations. Statistical analysis was carried out using the Student's *t*-test between 2 groups and one-way analysis of variance (ANOVA) among multiple groups. Tukey's post-hoc test was used for pairwise comparisons. Pearson's correlation analysis was performed to distinguish the correlation of two variates. Differences were considered statistically significant when $p \leq 0.05$.

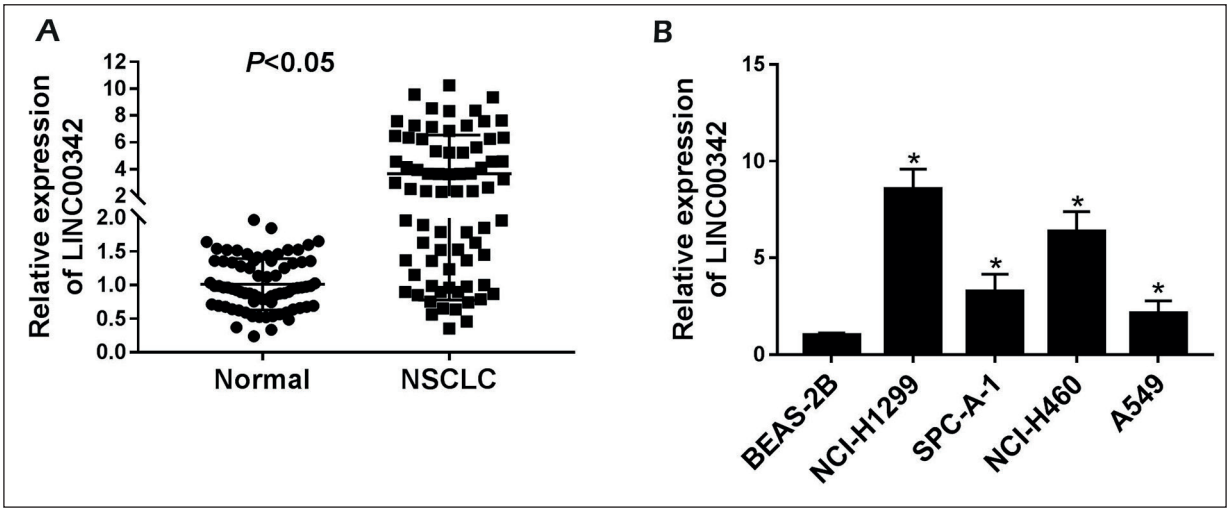

Figure 1. The expression of LINC00342 in NSCLC tissues and cell lines. ***A,*** Expression of LINC00342 in NSCLC tissues was higher than that in normal tissues (n=75). ***B,*** Expression of LINC00342 was increased in human lung cancer cell lines, including NCI-H1299, NCI-H460, SPC-A-1, and A549 compared with human bronchial epithelial cell line BEAS-2B. * $p<0.05$.

Results

LINC00342 was Up-Regulated in NSCLC Tissues and Cell Lines

To define the expression of LINC00342 in NSCLC tissues and cells, RT-qPCR was performed. As shown in Figure 1A, LINC00342 expression was markedly enhanced in NSCLC tissues compared with paired normal tissues. Meanwhile, LINC00342 was significantly increased in human lung cancer cell lines, including NCI-H1299, NCI-H460, SPC-A-1, and A549 rather than in human bronchial epithelial cell BEAS-2B (Figure 1B). Correlation analysis between the expression level of lncRNA LINC00342 and clinical indexes of NSCLC was exhibited in Table I. The data indicated that LINC00342 might play an oncogenic role in NSCLC.

LINC00342 Knockdown Suppressed Cell Proliferation, Colony Formation, Migration, and Invasion in NSCLC Cells

To determine the influence of LINC00342 in NSCLC *in vitro*, we knocked down LINC00342 expression and checked the efficiency of the knockdown. From the group of si-LINC00342-1, si-LINC00342-2, and si-LINC00342-3, it was notably observed that the expression of LINC00342 of si-LINC00342-3 group was decreased most in NCI-H1299 and NCI-H460 cells (Figure 2A).

Table I. Correlation analysis between the expression level of LncRNA LINC00342 and clinical indexes of non-small cell lung cancer.

Clinicopathological feature	LINC00342 expression (n)		*p*-value
	High	Low	
Age			> 0.05
< 55 years	17	19	
≥ 55 years	20	19	
Gender			> 0.05
Female	15	18	
Male	19	23	
Lymph node metastasis			< 0.05
Yes	28	13	
No	10	24	
TNM stage			< 0.05
I-II	27	12	
III	14	22	

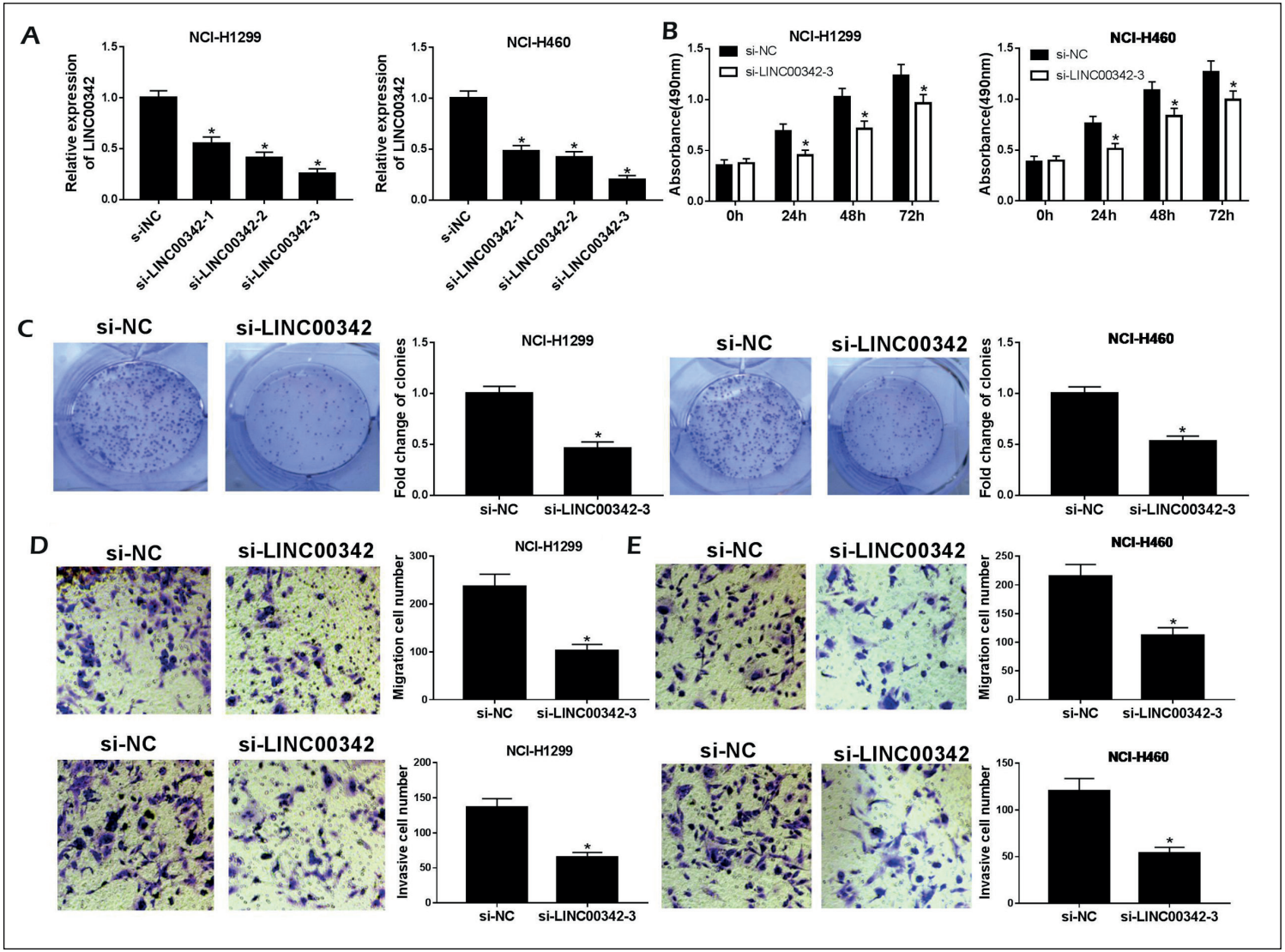

Figure 2. Knockdown of LINC00342 inhibited cell proliferation, colony formation, migration and invasion in NSCLC cells. ***A,*** Efficiency of LINC00342 knockdown was detected by qRT-PCR in NCI-H1299 and NCI-H460. ***B,*** MTT assay was used to examine cell proliferation of NCI-H1299 and NCI-H460 transfected with si-LINC00342-3. ***C,*** Colony formation assays were performed in si-LINC00342-3 transfected cells of NCI-H1299 and NCI-H460 (100×). ***D, E,*** Cell invasion and migration were measured by transwell both in NCI-H1299 and NCI-H460 transfected with si-LINC00342-3. * $p<0.05$ (100×).

Thus, si-LINC00342-3 was chosen for the following analysis. MTT assay revealed that the proliferation of NCI-H1299 and NCI-H460 cells transfected with si-LINC00342-3 was remarkably inhibited compared with si-NC group (Figure 2B). Colony formation assay showed that the number of colonies was pronouncedly declined in LINC00342 under-expressed NCI-H1299 and NCI-H460 cells compared with that in si-NC transfection cells (Figure 2C). The transwell analysis indicated that the number of migrated cells and invaded cells in NCI-H1299 containing si-LINC00342-3 group was strongly lower than that in si-NC group (Figure 2D). The case of cell migration and invasion in NCI-H460 (Figure 2E) was similar to the result in NCI-H1299. All data suggested that LINC00342 down-regulation prejudiced cell viability and metastasis in NSCLC *in vitro*.

LINC00342 Targeted MiR-203a-3p Directly in NSCLC

To examine the interaction of LINC00342 and miR-203a-3p, the online software DIANA was used to predict the potential binding sites between LINC00342 and miR-203a-3p. LINC00342 wt or mutant sequences were exhibited in Figure 3A and cloned into the luciferase reporter vector. Afterwards, LINC00342 wt or LINC00342 mut and miR-203a-3p mimic, miR-203a-3p mimic NC, miR-203a-3p antibody or miR-203a-3p antibody NC were transfected into NCI-H1299 and NCI-H460 cells. Relative luciferase activity was notably slumped both in NCI-H1299 and NCI-H460 with LINC00324-wt and miR-203a-3p mimic group compared with other groups (Figure 3B). Otherwise, the relative luciferase activity increased steeply in two cells, cotransfected with LINC00342-wt and anti-203a-3p rather than other items (Figure 3C). These data showed the interaction that LINC00342 targeted miR-203a-3p directly.

MiR-203a-3p was Weakly Expressed in NSCLC Tissues and Cell Lines, and its Expression was Regulated by LINC00342

To determine the possible role of miR-203a-3p in NSCLC, the expression of miR-203a-3p was checked by qRT-PCR first. As exhibited in Figure 4A, miR-203a-3p expression was prominently weakened in NSCLC tissues compared with adjacent normal tissues. Unsurprisingly, miR-203a-3p was down-regulated among NCI-H1299, NCI-H460, SPC-A-1, and A549 cell lines compared with BEAS-2B (Figure 4B). Correlation analysis found that the expression of miR-203a-3p was significantly negatively correlated with the expression of LINC00342 (Figure 4C). Next, we investigated the effect of LINC00342 overexpression and underexpression on the expression of miR-203a-3p. Both in NCI-H1299 and NCI-H460, pcDNA-LINC00342 transfection could diminish the expression of miR-203a-3p compared with pcDNA control transfection, while si-LINC00342-3 availably reinforced miR-203a-3p expression compared with si-NC (Figure 4D).

LINC00342 Induced Cell Proliferation, Colony Formation, Migration, and Invasion Through Regulating MiR-203a-3p Expression by Targeting it

To further explore the molecular mechanism of LINC00342, contributing to the cancer processes in NSCLC, several researches were performed. First, we detected the expression of miR-203a-3p in different groups. The expression of miR-203a-3p was increased rapidly when cells were transfected with miR-203a-3p mimic compared with NC. Meanwhile, a weakened expression of miR-203a-3p occurred in NCI-H1299 and NCI-H460 transfected with miR-203a-3p mimic and pcDNA-LINC00342 compared with miR-203a-3p+pcDNA control (Figure 5A). MTT assay revealed that miR-203a-3p mimic inhibited cell proliferation at 24, 48, and 72 h after transfection both in NCI-H1299 and NCI-H460, while miR-203a-3p mimic and pcDNA-linc00342 co-transfection reversed this inhibition on cell proliferation (Figure 5B). Colony formation assay stated that the colonies in NCI-H1299 and NCI-H460 cells transfected with miR-203a-3p decreased markedly. Whereas, the number of colonies restored in cells transfected with miR-203a-3p mimic and pcDNA-LINC00342 compared with control (Figure 5C). As for cell migration, miR-203a-3p mimic had the ability to absent the number of migrated cells compared with NC; however, LINC00342 up-regulating synchronously could make the number of migrated cells recovered compared with control (Figure 5D). The consequence of cell invasion was consistent with the result of cell migration (Figure 5E). All data above hinted that LINC00342 regulated cell proliferation, colony formation, migration, and invasion via binding to miR-203a-3p competitively.

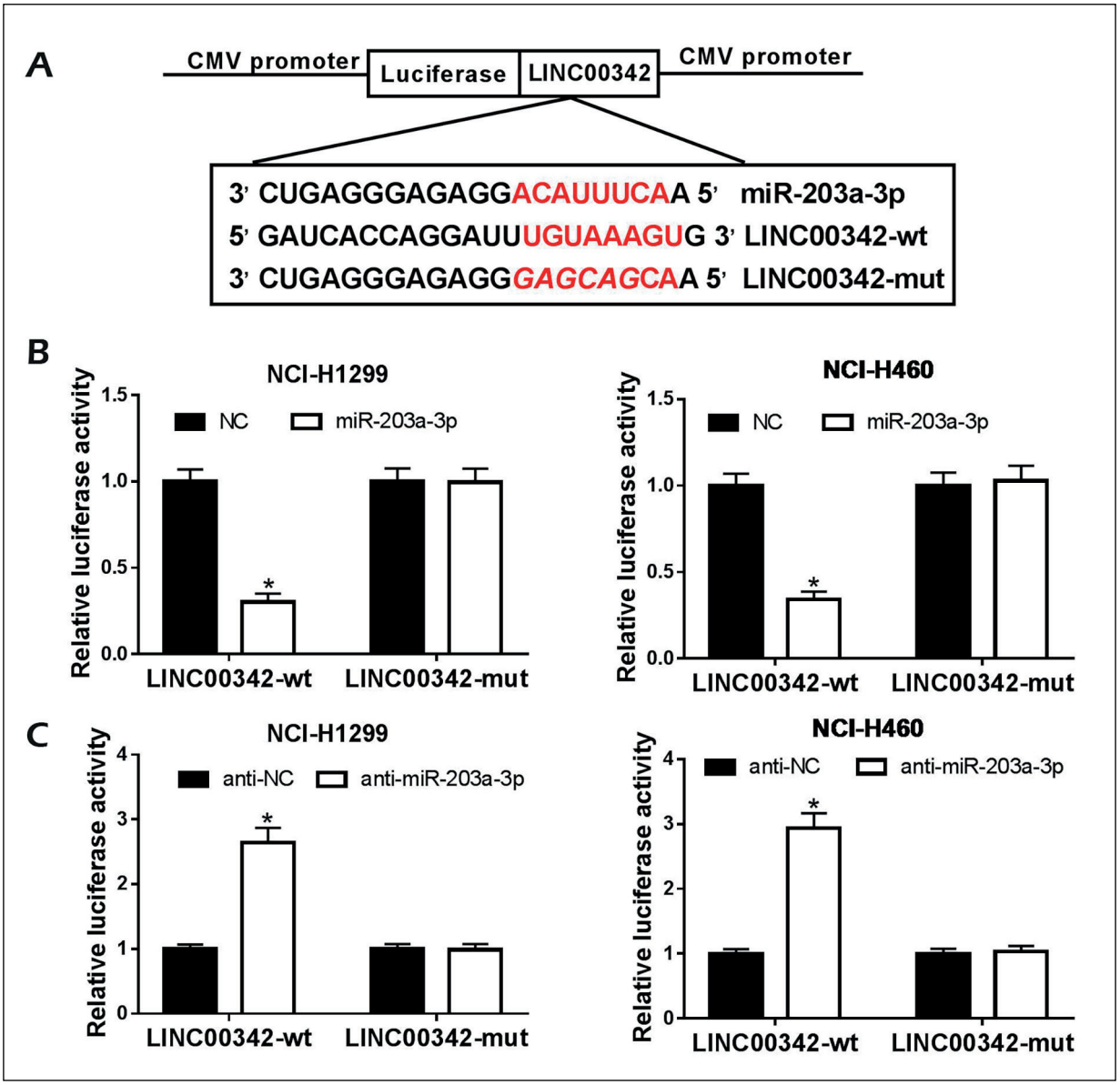

Figure 3. The interaction of LINC00342 and miR-203a-3p. ***A***, Binding sites were predicted by online software DIANA. ***B***, ***C***, Luciferase reporter assay was carried out to confirm the relationship between LINC00342 and miR-203a-3p in NCI-H1299 and NCI-H460 transfected with different groups (LINC00342-wt+miR-203a-3p mimic, LINC00342-mut+miR-203a-3p mimic, LINC00342-wt+anti-miR-203a-3p mimic, LINC00342-mut+anti-miR-203a-3p mimic and control). * p <0.05.

Discussion

NSCLC is the most common subtype of lung cancer. Unsatisfactory survival rates derive from challenges in advanced diagnosis and prevention[14]. Molecular alterations characterizing NSCLC are multiple, and they play a role in networks that cause molecular imbalances producing a malignant phenotype. These modifications include changes in gene expression, copy number changes, different methylation patterns, and expression changes of non-coding RNA[15,16]. We showed that specific expression patterns of lncRNAs can be used as potential biomarkers in biology, diagnosis, and therapy of NSCLC[17]. Therefore, new biomarker excavations for early diagnosis and potential therapeutic targets can significantly improve clinical outcomes and survival rates of lung cancer patients.

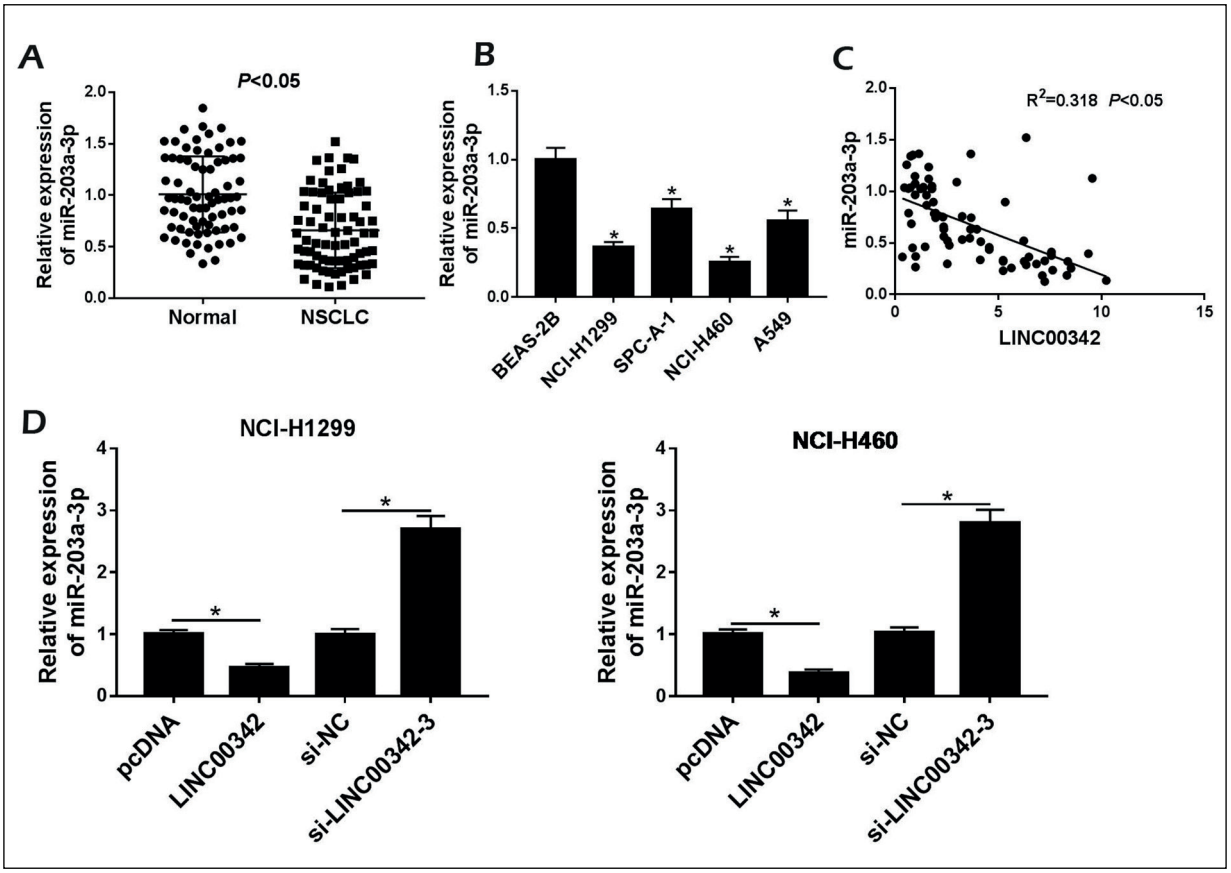

Figure 4. The expression of miR-203a-3p was measured in NSCLC tissues and cell lines, and its relationship with LINC00342 expression. ***A,*** MiR-203a-3p was down-regulated in NSCLC tissues compared with normal tissues (n=75). ***B,*** Expression of miR-203a-3p was reduced significantly in human lung cancer cell lines (NCI-H1299, NCI-H460, SPC-A-1, and A549) compared with human bronchial epithelial cell line BEAS-2B. ***C***, Expression of miR-203a-3p was negatively correlated with LINC00342 expression. ***D***, Relative expression of miR-203a-3p was measured in NCI-H1299 and NCI-H460 cells containing different groups (pcDNA-LINC00342, pcDNA empty vector, si-LINC00342, and si-NC). * $p<0.05$.

The previous study identified a remarkably up-regulated lncRNA LINC00342 from the expression profile of lncRNA in NSCLC by re-annotation microarray dataset. In addition, they proved that LINC00342 could suppressed cell proliferation *in vitro*[18]. Consistently, our result also suggested that LINC00342 expression was enhanced, suggesting its carcinogenic role in NSCLC. Tang et al[9] indicated that higher LINC00342 expression was relevant to poor overall survival and LINC00342 promoted cell viability by inhibiting the expression of p53 and PTEN proteins. In our study, we focused on the function of LINC00342 through a series of assays, such as cell proliferation, invasion, and migration. The result showed that knockdown of LINC00342 weakened the cell growth and metastasis of NSCLC, which verified LINC00342 might be a putative biomarker for the treatment of NSCLC. Additionally, the relation between LINC00342 and miR-203a-3p was indicated, and miR-203a-3p exerted its role through targeted by LINC00342. MiR-203a-3p acting as tumor inhibitor has been introduced in numerous cancers; for example, the expression of miR-203a-3p was decreased in CRC tissues, and miR-203a-3p overexpression led to inhibited cell proliferation and reduced chemoresistance[20]. In nasopharyngeal carcinoma (NPC), miR-203a-3p was down-regulated in NPC tissues and cell lines and overexpression of miR-203a-3p suppressed xenograft tumor growth and lung metastasis *in vivo*, suggesting its anticancer role in NSCLC[21]. Circular RNA circTADA2A could readily target miR-203a-3p to up-regulate the expression of CREB3, leading to tumorigenesis and metastasis *in vivo*[22]. In this research, the expression of miR-203a-3p was declined significantly in NSCLC

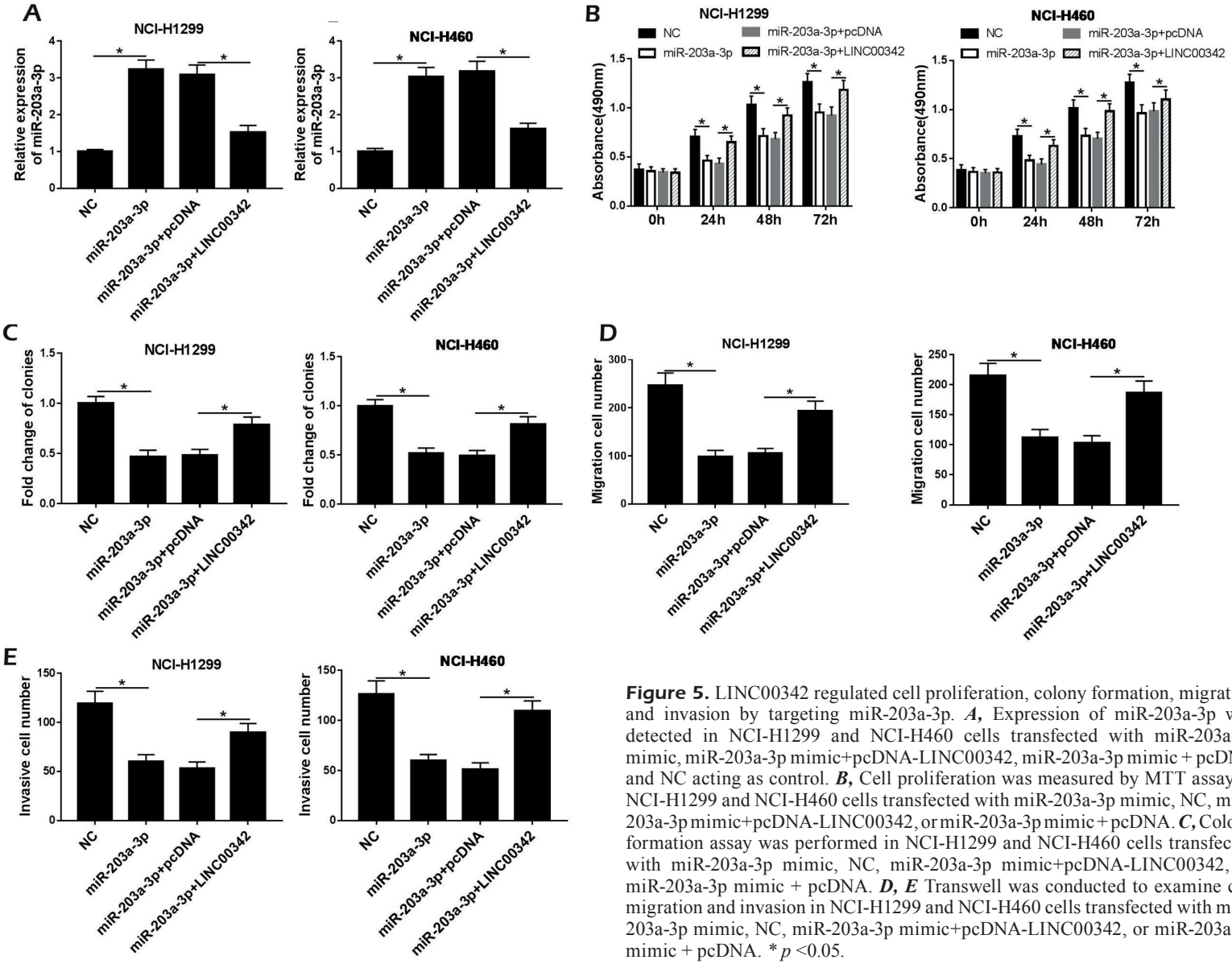

Figure 5. LINC00342 regulated cell proliferation, colony formation, migration and invasion by targeting miR-203a-3p. ***A,*** Expression of miR-203a-3p was detected in NCI-H1299 and NCI-H460 cells transfected with miR-203a-3p mimic, miR-203a-3p mimic+pcDNA-LINC00342, miR-203a-3p mimic + pcDNA and NC acting as control. ***B,*** Cell proliferation was measured by MTT assay of NCI-H1299 and NCI-H460 cells transfected with miR-203a-3p mimic, NC, miR-203a-3p mimic+pcDNA-LINC00342, or miR-203a-3p mimic + pcDNA. ***C,*** Colony formation assay was performed in NCI-H1299 and NCI-H460 cells transfected with miR-203a-3p mimic, NC, miR-203a-3p mimic+pcDNA-LINC00342, or miR-203a-3p mimic + pcDNA. ***D, E*** Transwell was conducted to examine cell migration and invasion in NCI-H1299 and NCI-H460 cells transfected with miR-203a-3p mimic, NC, miR-203a-3p mimic+pcDNA-LINC00342, or miR-203a-3p mimic + pcDNA. * $p < 0.05$.

tissues and cell lines, consistently with the previous work mentioned. Moreover, miR-203a-3p, with several binding sites for LINC00342, was identified by bioinformatics analysis online. Luciferase assays exhibited authentic interactions between miR-203a-3p and LINC00342, providing a possible regulatory mechanism axis of the development and metastasis in NSCLC.

Conclusions

The expression of LINC00342 was strongly increased in NSCLC tissues and cell lines. Function analysis showed that LINC00342 knockdown inhibited cell proliferation, invasion, and migration. Furthermore, the truth that LINC00342 contributes to cell growth and metastasis by targeting miR-203a-3p in NSCLC was first demonstrated in this study, providing potential potency as a NSCLC-targeted therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
2) Morgensztern D, Ng SH, Gao F, Govindan R. Trends in stage distribution for patients with non-small cell lung cancer: a national cancer database survey. J Thorac Oncol 2010; 5: 29-33.
3) Helissey C, Champiat S, Soria JC. Immune checkpoint inhibitors in advanced nonsmall cell lung cancer. Curr Opin Oncol 2015; 27: 108-117.
4) Verdecchia A, Francisci S, Brenner H, Gatta G, Micheli A, Mangone L Kunkler I; EUROCARE-4 Working Group. Recent cancer survival in Europe: a 2000-02 period analysis of EUROCARE-4 data. Lancet Oncol 2007; 8: 784-796.
5) Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
6) Yang YR, Zang SZ, Zhong CL, Li YX, Zhao SS, Feng XJ. Increased expression of the lncRNA PVT1 promotes tumorigenesis in non-small cell lung cancer. Int J Clin Exp Pathol 2014; 7: 6929-6935.
7) Xie X, Liu HT, Mei J, Ding FB, Xiao HB, Hu FQ, Hu R, Wang MS. LncRNA HMlincRNA717 is down-regulated in non-small cell lung cancer and associated with poor prognosis. Int J Clin Exp Pathol 2014; 7: 8881-8886.
8) Nie W, Ge HJ, Yang XQ, Sun X, Huang H, Tao X, Chen WS, Li B. LncRNA-UCA1 exerts oncogenic functions in non-small cell lung cancer by targeting miR-193a-3p. Cancer Lett 2016; 371: 99-106.
9) Makarova JA, Kramerov DA. Noncoding RNAs. Biochemistry (Mosc) 2007; 72: 1161-1178.
10) Hata A, Kashima R. Dysregulation of microRNA biogenesis machinery in cancer. Crit Rev Biochem Mol Biol 2016; 51: 121-134.
11) Feng H, Ge F, Du L, Zhang Z, Liu D. MiR-34b-3p represses cell proliferation, cell cycle progression and cell apoptosis in non-small-cell lung cancer (NSCLC) by targeting CDK4. J Cell Mol Med 2019; 23: 5282-5291.
12) Wang G, Han J, Zhuang L, Li S, Gong Q, Chen Y. Serum starvation induces cell death in NSCLC via miR-224. Onco Targets Ther 2019; 12: 3953-3962.
13) Meng H, Huang Q, Zhang X, Huang J, Shen R, Zhang B. MiR-449a regulates the cell migration and invasion of human non-small cell lung carcinoma by targeting ADAM10. Onco Targets Ther 2019; 12: 3829-3838.
14) Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JH, Beasley MB, Chirieac LR, Dacic S, Duhig E, Flieder DB, Geisinger K, Hirsch FR, Ishikawa Y, Kerr KM, Noguchi M, Pelosi G, Powell CA, Tsao MS, Wistuba I; WHO Panel. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol 2015; 10: 1243-1260.
15) Pikor LA, Ramnarine VR, Lam S, Lam WL. Genetic alterations defining NSCLC subtypes and their therapeutic implications. Lung Cancer 2013; 82: 179-189.
16) Bica-Pop C, Cojocneanu-Petric R, Magdo L, Raduly L, Gulei D, Berindan-Neagoe I. Overview upon miR-21 in lung cancer: focus on NSCLC. Cell Mol Life Sci 2018; 75: 3539-3551.
17) Ricciuti B, Mencaroni C, Paglialunga L, Paciullo F, Crinò L, Chiari R, Metro G. Long noncoding RNAs: new insights into non-small cell lung cancer biology, diagnosis and therapy. Med Oncol 2016; 33: 18.
18) Wang L, Chen Z, An L, Wang Y, Zhang Z, Guo Y. Analysis of long non-coding rna expression profiles in non-small cell lung cancer. Cell Physiol Biochem 2016; 38: 2389-2400.

19) Tang H, Zhao L, Li M, Li T, Hao Y. Investigation of LINC00342 as a poor prognostic biomarker for human patients with non-small cell lung cancer. J Cell Biochem 2019; 120: 5055-5061.

20) Xiao Z, Qu Z, Chen Z, Fang Z, Zhou K, Huang Z, Guo X, Zhang Y. LncRNA HOTAIR is a prognostic biomarker for the proliferation and chemoresistance of colorectal cancer via MiR-203a-3p-mediated Wnt/ss-catenin signaling pathway. Cell Physiol Biochem 2018; 46: 1275-1285.

21) Jiang N, Jiang X, Chen Z, Song X, Wu L, Zong D, Song D, Yin L, Wang D, Chen C, Bian X, He X. MiR-203a-3p suppresses cell proliferation and metastasis through inhibiting LASP1 in nasopharyngeal carcinoma. J Exp Clin Cancer Res 2017; 36: 138.

22) Wu Y, Xie Z, Chen J, Chen J, Ni W, Ma Y, Huang K, Wang G, Wang J, Ma J, Shen S, Fan S. Circular RNA circTADA2A promotes osteosarcoma progression and metastasis by sponging miR-203a-3p and regulating CREB3 expression. Mol Cancer 2019; 18: 73.