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LINC01116 promotes proliferation, invasion and migration of osteosarcoma cells by silencing p53 and EZH2

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Abstract. – OBJECTIVE: The aim of this study was to elucidate the expression pattern and potential function of LINC01116 in regulating the progression of osteosarcoma.

PATIENTS AND METHODS: Expression levels of LINC01116 in osteosarcoma tissues (n=52) and adjacent normal tissues (n=52) were detected by quantitative Real-time polymerase chain reaction (qRT-PCR). Survival analysis and univariate analysis were performed in osteosarcoma patients based on the relative expression levels of LINC01116 and clinical data. Overexpression or silence of LINC01116 in osteosarcoma cells was achieved by transfection of plasmid complementary deoxyribonucleic acid (pcDNA)-LINC01116 or si-LINC01116, respectively. Subsequently, the regulatory effects of LINC01116 on cellular behaviors of osteosarcoma cells were examined by cell counting kit-8 (CCK-8), transwell and flow cytometry. Meanwhile, the potential mechanism of LINC01116 in regulating the progression of osteosarcoma was explored by RNA binding protein immunoprecipitation (RIP), chromatin immunoprecipitation (ChIP) and Western blot. Potential target genes in osteosarcoma were searched, and their functions were clarified through a series of rescue experiments.

RESULTS: LINC01116 expression in osteosarcoma tissues was significantly higher than adjacent normal tissues. The expression of LINC01116 was negatively correlated with overall survival, whereas positively correlated with tumor size and clinical grade of osteosarcoma patients. Transfection of pcDNA-LINC01116 significantly enhanced proliferative, migratory and invasive abilities of U2OS cells, shortened G0/G1 phase period, and inhibited cell apoptosis. However, transfection of si-LINC01116 in MG63 cells obtained the opposite trends in the above-mentioned cellular behaviors. Furthermore, RIP assay confirmed the binding of enhancer of zeste homolog 2 (EZH2) to LINC01116. Knockdown of LINC01116 significantly up-regulated the expressions of phosphatase and tensin homolog deleted on chromosome ten (PTEN) and p53. Moreover, EZH2 knockdown could reverse the inhibitory effect of LINC01116 on carcinogenesis of osteosarcoma.

CONCLUSIONS: LINC01116 is highly expressed in osteosarcoma. Up-regulated LINC01116 can promote cell proliferation, invasion and cell cycle progression, while inhibiting the apoptosis of osteosarcoma cells. Furthermore, LINC01116 is involved in the development of osteosarcoma by binding to EZH2 to regulate expressions of PTEN and p53.

Key Words:
Osteosarcoma, LINC01116, EZH2, PTEN, P53.

Introduction

Osteosarcoma (OS) is the most common primary malignancy of bone tissue in children and young adults. OS is originated from mesenchymal cells. Meanwhile, its pathological features include the presence of malignant mesenchymal spindle cells and bone matrix production[1]. Although the incidence of OS is not very high[2], it often occurs in the extremities and easily metastasizes to lung[3]. Unfortunately, the clinical outcome of OS has not been greatly improved within the past 30 years. Moreover, cenetic and biological complexity of OS hinders its progression in clinical treatment. Therefore, it is necessary to comprehensively elucidate the pathogenesis of OS. Early-stage defects in deoxyribonucleic acid (DNA) repair/monitoring are one of the major factors responsible for the occurrence and aneuploidy of OS[4]. Gene mu-

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tations in OS are complex, which differ greatly in different individuals. Unlike other sarcomas, OS lacks typical translocations or genetic mutations[5]. It is also well-known that OS is a malignant tumor represented by a wide range of abnormalities and isomerism in chromosome numbers and structures. Long noncoding RNAs (lncRNAs) are a type of non-coding RNAs with over than 200 nt in length[6]. LncRNA is dysregulated in diseases, especially in tumors. It is capable of regulating pathological processes, such as cell proliferation, apoptosis, necrosis and autophagy[7,8]. Several lncRNAs have been identified to be involved in the progression of OS[9]. Wang G. et al[10] have indicated that some of the classical lncRNAs are up-regulated in OS, which promote disease progression and are associated with poor prognosis. However, the specific mechanism of lncRNAs in the pathogenesis of OS remains unclear. Previously, it has been reported that LINC01116 is abnormally up-regulated in different tumors. For example, LINC01116 is over-expressed in prostate cancer and down-regulated after sulforaphane treatment[11]. In hepatocellular carcinoma, LINC01116 is a potential risk factor closely related to cancer development[12]. Therefore, clarification of OS-specific lncRNAs contributes to completely reveal the pathogenesis of OS and improve clinical outcomes.

Patients and Methods

Tissue Collection

52 paired OS tissues (2×2×2 cm in size) and adjacent normal tissues (2-3 cm away from tumor edge, 2×2×2 cm in size) were harvested from OS patients who received treatment in the Second Hospital of Tianjin Medical University. Tissue samples were immediately placed in liquid nitrogen and preserved at -80°C. The experiment was approved by the Medical Ethics Committee of The Second Hospital of Tianjin Medical University. Informed consent was obtained from patients before the study.

RNA Extraction

Cells or tissues were lysed with TRIzol (Invitrogen, Carlsbad, CA, USA) reagent, followed by incubation with 200 μL of chloroform. After gentle mixture and centrifugation, the upper aqueous phase was incubated with 600 μL of isopropanol. After centrifugation, the precipitate was washed with 70% ethanol and centrifuged again. Collected precipitate was then air-dried and diluted in RNase-free water (Beyotime, Shanghai, China). After quantification and purification of total RNA samples, they were sub-packed and preserved at -80°C for use.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was reversely transcribed into complementary deoxyribonucleic acid (cDNA) using PrimeScript RT Master Mix (TaKaRa, Otsu, Shiga, Japan). Quantitative Real-time polymerase chain reaction (qRT-PCR) was conducted with SYBR Green method to determine the relative expression levels of target genes. Primer sequences used in this study were as follows: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (F: 5'-CACCCACTCCTCCACCTTTG-3', R: 5'-CCACCACCCTGTTGCTGTAG-3'); U6 (F: 5'-GGGAGATACCATGATCACGAAGGT-3', R: 5'-CCACAAATTATGCAGTCGAGTTTCCC-3'); LINC01116 (F: 5'-GAATGGCAAAGCACTTGGGG-3', R: 5'-AGCTCTCCTTGCAGGTAGGT-3'); Phosphatase and tensin homolog deleted on chromosome ten (PTEN) (F: 5'-TGGATTCGACTTAGACTTGACCT-3', R: 5'-GGTGGGTTATGGTCTTCAAAAGG-3'); P53 (F: 5'-CAGCACATGACGGAGGTTGT-3', R: 5'-TCATCCAAATACTCCACACGC-3'); Enhancer of zeste homolog 2 (EZH2) (F: 5'-AATCAGAGTACATGCGACTGAGA-3', R: 5'-GCTGTATCCTTCGCTGTTTCC-3').

Construction of pcDNA-LINC01116

According to the sequence analysis of LINC01116, single-chain oligo of LINC01116 was designed and synthesized. Meanwhile, the splicing reaction was carried out by qRT-PCR. Ligated PCR product was cloned into T vector, followed by insertion into the host strain. After sequencing verification, the synthesized target fragment was cloned into destination vector pcDNA3.1 to obtain pcDNA-LINC01116.

Cell Culture and Transfection

OS cell lines (Saos2, HOS, U2OS and MG63) and normal human osteoblast cell lines (hFOB1.19) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM: Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS: Gibco, Rockville, MD, USA), and maintained in a 37°C, 5% CO_2 incubator. Cells were then seeded into 6-well plates

at a density of 1×10^4 cells/well. Cell transfection was performed until 75-85% of confluence using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Fresh medium was replaced at 6 h. Transfection plasmid sequences were: si-LINC01116: AAAGACACTGGAAGAATACGTGAAT; si-NC: UUCUCCGAACGUGUCACGUTT.

Subcellular Distribution Analysis

Cells were digested, washed with phosphate-buffered saline (PBS) and incubated with 500 μL of cell fractionation buffer on ice for 10 min. After centrifugation, nuclei were formed as precipitate, followed by incubation with cell fractionation buffer, cell disruption buffer and lysis binding solution. Subsequently, the supernatant containing cytoplasm was incubated with lysis binding solution. After mixing with absolute ethanol and incubation with wash solution, the precipitate and supernatant were centrifuged, eluded using elution solution, and quantified.

Cell Counting Kit-8 (CCK-8) Assay

Cells were first seeded into 96-well plates (Corning, Corning, NY, USA) at a density of 2×10^4 cells per well. At appointed time points, fresh medium containing 10 μL of CCK-8 solution (Beyotime, Shanghai, China) was added into each well, followed by incubation for 2 h in dark. Finally, absorbance at 450 nm was detected using a microplate reader, and the growth curve was plotted.

Cell Apoptosis Determination

Transfected cells for 48 h were digested, and incubated with 10 μL of AnnexinV and 5 μL of PI (propidium iodide) for 15 min in dark. Before determination, the cells were incubated with 350 μL of binding buffer in dark for 30 min. Finally, cell apoptosis was analyzed by a BD FACSCalibur flow cytometer (BD Bioscience, Franklin Lakes, NJ, USA) within 1 h.

Cell Cycle Determination

Cells were digested, centrifuged at 800 rpm for 5 min and fixed in pre-cooled 70% ethanol at -20°C overnight. On the next day, cells were incubated with 10 μL of RNase for 30 min and dyed with PI for 15 min. Finally, flow cytometer determination was performed.

Transwell Migration and Invasion Assays

OS cells were first prepared into 1×10^6/mL cell suspension. 100 μL of cell suspension and 700 μL of complete medium were added to the basolateral and apical chamber, respectively. 12 h later, Transwell chamber (Corning, Corning, NY, USA) was taken out and the medium was removed. Cells were fixed with 4% paraformaldehyde (Beyotime, Shanghai, China) and stained with crystal violet dye. Images were taken under a microscope (magnification 20×), and the number of migratory cells was calculated. Procedures of cell invasion assay were the same as the above-mentioned except for 100 μL of Matrigel (Corning, Corning, NY, USA) pre-coated in transwell chamber.

RNA Immunoprecipitation (RIP)

Cells were first lysed and re-suspended in Wash Buffer on ice. A protein-RNA complex was captured and digested with proteinase K to extract RNA. After that, purification was performed using phenol, chloroform, Salt Solution I, Salt Solution II, Precipitate Enhancer and RNase-free anhydrous ethanol. RNA was diluted in 10-20 μL of DEPC (Beyotime, Shanghai, China) water and preserved at -80°C for use. Finally, extracted RNA was subjected to qRT-PCR detection.

Chromatin Immunoprecipitation (ChIP)

Cells were cross-linked with 1% formaldehyde for 10 min at room temperature. Subsequently, cross-linked cells were lysed, and incubated with ChIP Dilution Buffer, PIC and Protein A Agarose/Salmon Sperm DNA (Millipore, Billerica, MA, USA). After centrifugation, 20 μL of the supernatant was collected as input. After incubation with 1 μL of antibody overnight at 4°C, the supernatant was cultured in 60 μL of Protein A Agarose/Salmon Sperm DNA. Immunoprecipitation complex was washed, eluted, and cross-linked overnight at 65°C. Finally, DNA fragments were subjected to PCR.

Western Blot

Total protein in cells was extracted using radio immunoprecipitation assay (RIPA) containing phenylmethanesulfonyl fluoride (PMSF) (Beyotime, Shanghai, China). Protein samples were separated by electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland). After blocking with 5% skim milk for 2 hours, the membranes were incubated with primary antibodies at 4°C overnight. On the next day, the membranes were incubated with corresponding secondary antibodies for 2 hours. Immuno-reactive bands were exposed by enhanced chemiluminescence (ECL) method and analyzed by Image J Software (NIH, Bethesda, MD, USA).

Table I. Correlation between LINC01116 expression with clinicopathological features of OS patients (n=52).

Clinicopathologic features	No. of cases	LINC01116 expression		*p*-value
		Low (n=26)	High (n=26)	
Age (years)				0.4022
≤ 20	29	13	16	
> 20	23	13	10	
Gender				0.7814
Male	27	14	13	
Female	25	12	13	
Tumor size				0.0260*
≤ 6 cm	24	16	8	
> 6 cm	28	10	18	
WHO grade				0.0091*
I-II	28	19	9	
III	25	8	17	
Pulmonary metastasis				0.0120*
Yes	23	16	7	
No	29	10	19	

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA) was used for all statistical analysis. Data were expressed as mean ± standard deviation (SD). *t*-test was used to analyze the difference between two groups. Survival was evaluated by Kaplan-Meier method. Chi-square test was used to analyze the correlation between LINC01116 expression and clinical data of OS patients. $p<0.05$ was considered statistically significant.

Results

High Expression of LINC01116 in OS

We first detected LINC01116 expression in OS tissues (n=52) and adjacent normal tissues (n=52) by qRT-PCR. Results showed that LINC01116 was highly expressed in OS tissues (Figure 1A). High expression of LINC01116 was associated with tumor size, clinical grade, and distant lung metastasis, whereas not correlated with age and gender (Table I). Subsequently, enrolled OS patients were assigned into high-level group and low-level group based on the expression levels of LINC01116 (Figure 1B). Overall survival of OS patients in high-level group was significantly shorter than that of patients in low-level group (Figure 1E). Moreover, LINC01116 expression in OS patients with grade III or tumor size > 6 cm was remarkably higher than those with grade I-II or tumor size ≤ 6 cm (Figure 1C, 1D). These data indicated the involvement of LINC01116 in OS development.

LINC01116 Expression in OS Cell Lines

To investigate the regulatory effect of LINC01116 on OS cells, we detected LINC01116 expression in osteoblast cell line (hFOB1.19) and OS cell lines (Saos2, HOS, U2OS and MG63) by qRT-PCR as well. Results demonstrated that LINC01116 was highly expressed in OS cells (Figure 2A). Since LINC01116 expression was relatively high in MG63 cells and low in U2OS cells, these two cell lines were selected for subsequent experiments. Transfection of si-LINC01116 markedly down-regulated LINC01116 expression in MG63 cells, while transfection of pcDNA-LINC01116 significantly up-regulated LINC01116 expression in U2OS cells (Figure 2B). Next, we separated nuclear and cytoplasmic fractions of MG63 and U2OS cells. QRT-PCR data revealed that LINC01116 was mainly distributed in cytoplasm, indicating its function at post-transcriptional level (Figure 2C).

Overexpression of LINC01116 Accelerated the Proliferation, Invasion and Migration of OS Cells

CCK-8 assay indicated that the viability of MG63 cells transfected with si-LINC01116 at 24, 48, 72, and 96 h was markedly reduced when compared with that of controls (Figure 3A). Transfection of si-LINC01116 induced the apoptosis of MG63 cells (Figure 3B). Meanwhile, G0/G1 phase prolonged and S phase shortened in MG63 cells with LINC01116 knockdown relative

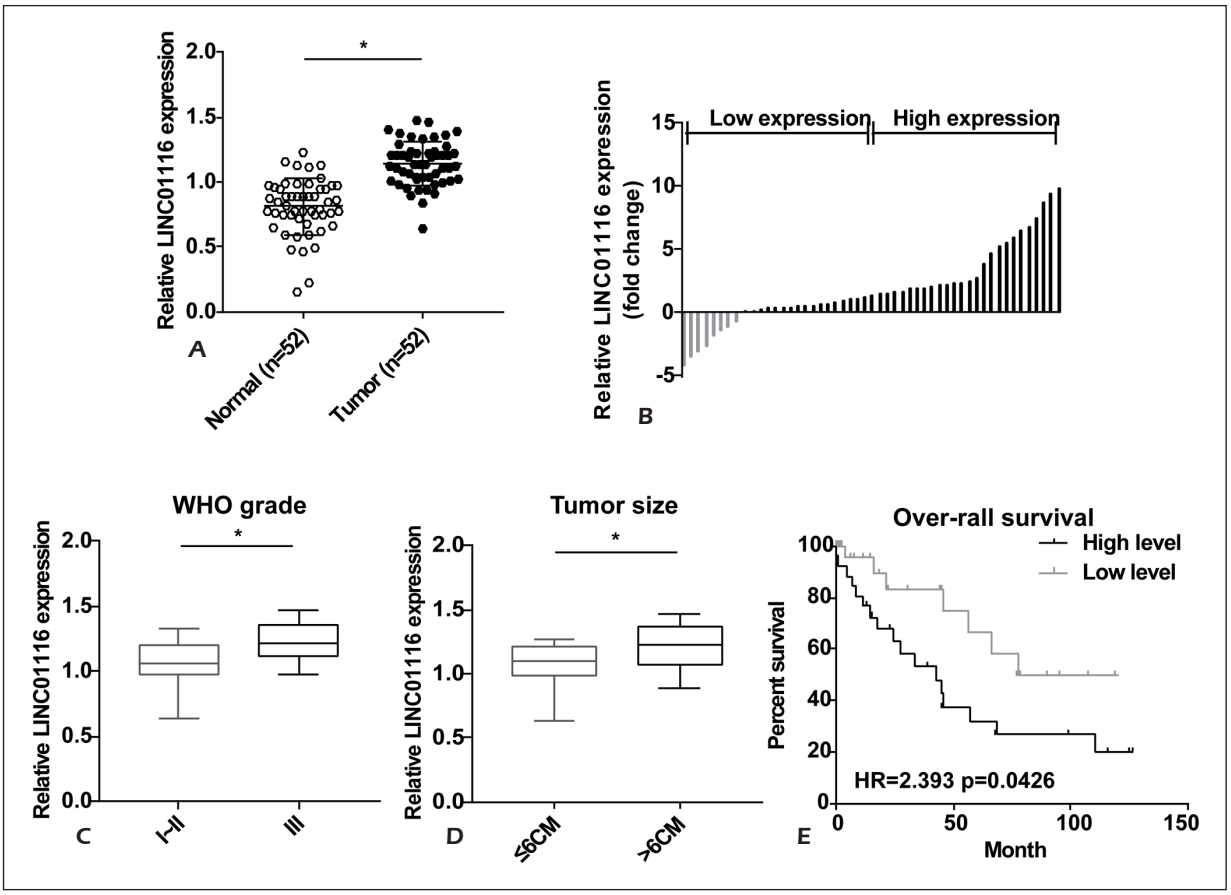

Figure 1. High expression of LINC01116 in OS patients. ***A-B***, LINC01116 expression was significantly higher in OS tissues (n=52) than adjacent normal tissues (n=52) detected by qRT-PCR. ***C***, LINC01116 expression was significantly higher in grade III OS patients than those in grade I-II detected by qRT-PCR. ***D***, LINC01116 expression was remarkably higher in patients with tumor size > 6 cm those with tumor size ≤ 6 cm detected by qRT-PCR. ***E***, Overall survival of OS patients in high-level group was significantly shorter relative to those in low-level group.

to those of controls (Figure 3C). Overexpression of LINC01116 in U2OS cells obtained the opposite results in cell proliferation, apoptosis and cell cycle progression (Figure 3D-3F). Invasive and migratory potentials of OS cells were evaluated by Transwell assay. The results suggested that the migratory and invasive abilities were significantly inhibited in MG63 cells transfected with si-LINC01116 (Figure 3G). Conversely, LINC01116 overexpression markedly accelerated U2OS cells to migrate and invade (Figure 3H). To sum up, LINC01116 promoted the proliferation, migration and invasion of OS cells, whereas inhibited cell apoptosis.

LINC01116 Silenced p53 and PTEN by Binding to EZH2

Generally, lncRNA participates in tumorigenesis by binding to specific RNA-binding proteins. Here, we detected the interaction between LINC01116 and potential RNA-binding proteins that regulate transcriptional levels of OS cells by RIP assay. The data showed that LINC01116 was directly integrated with EZH2 (Figure 4A). We then focused on p53 and PTEN, which were the potential targets of EZH2 with tumor-suppressor effect for the following researches. Expressions of p53 and PTEN were negatively regulated by LINC01116 in MG63 cells at both mRNA and protein levels (Figure 4B, 4C). Hence, we believed that p53 and PTEN might be the targets of LINC01116. Next, we constructed si-EZH2 to further elucidate the potential functions of EZH2, p53 and PTEN in OS development. Transfection of si-EZH2 significantly up-regulated the mRNA and protein levels of p53 and PTEN (Figure 4D, 4E). ChIP assay was conducted to determine whether LINC01116 could inhibit the expressions

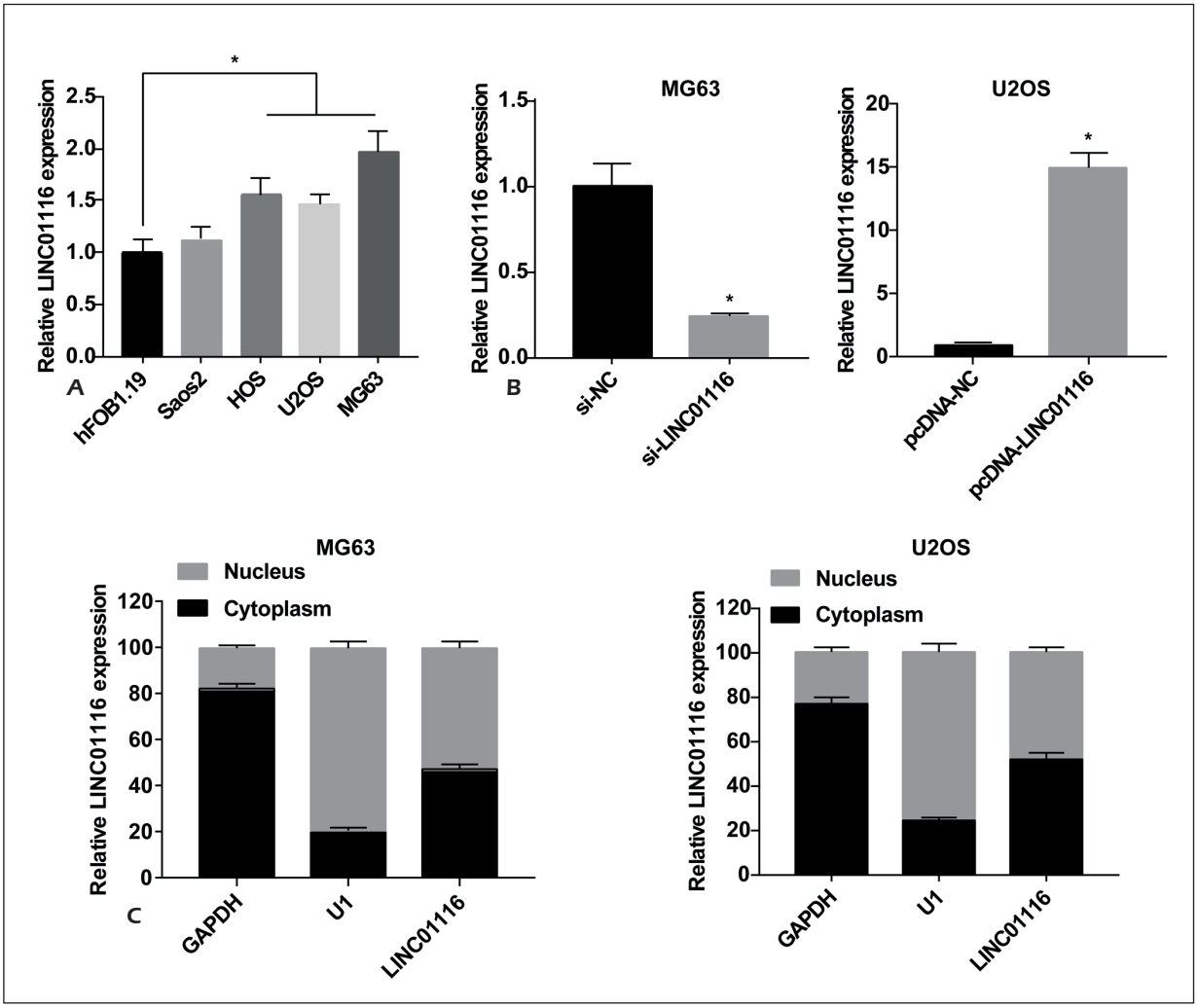

Figure 2. LINC01116 expression in OS cell lines. ***A***, LINC01116 was highly expressed in OS cells (Saos2, HOS, U2OS and MG63) relative to osteoblast cell line (hFOB1.19) detected by qRT-PCR. ***B***, Transfection of si-LINC01116 markedly downregulated LINC01116 expression in MG63 cells detected by qRT-PCR. Transfection of pcDNA-LINC01116 significantly upregulated LINC01116 expression in U2OS cells detected by qRT-PCR. ***C***, LINC01116 was mainly distributed in cytoplasm of MG63 and U2OS cells.

of PTEN and p53 by interacting with EZH2. As the data revealed, LINC01116 knockdown obviously decreased the binding capacities of PTEN and p53 to EZH2 (Figure 4F, 4H). Moreover, LINC01116 knockdown promoted demethylation modification of H3K4me2 in p53 and PTEN (Figure 4G, 4I). Our results suggested that EZH2 directly bound to promoter regions of PTEN and p53 and mediated H3K4me2 demethylation modification. All the above data demonstrated that LINC01116 participated in OS development by down-regulating PTEN and p53 through binding to EZH2.

LINC01116 Exerted its Role in Regulating OS Cells Through Down-Regulating EZH2

Rescue experiments were finally conducted to evaluate the role of LINC01116/EZH2 axis in OS development. In this study, MG63 cells were transfected with si-NC, si-LINC01116 or si-LINC01116+-si-EZH2, respectively. CCK-8 assay revealed that significantly inhibited cell viability caused by LINC01116 knockdown could be partially reversed by EZH2 knockdown (Figure 5A). Induced apoptosis of MG63 cells transfected with si-LINC01116 was inhibited to some extent after co-transfection

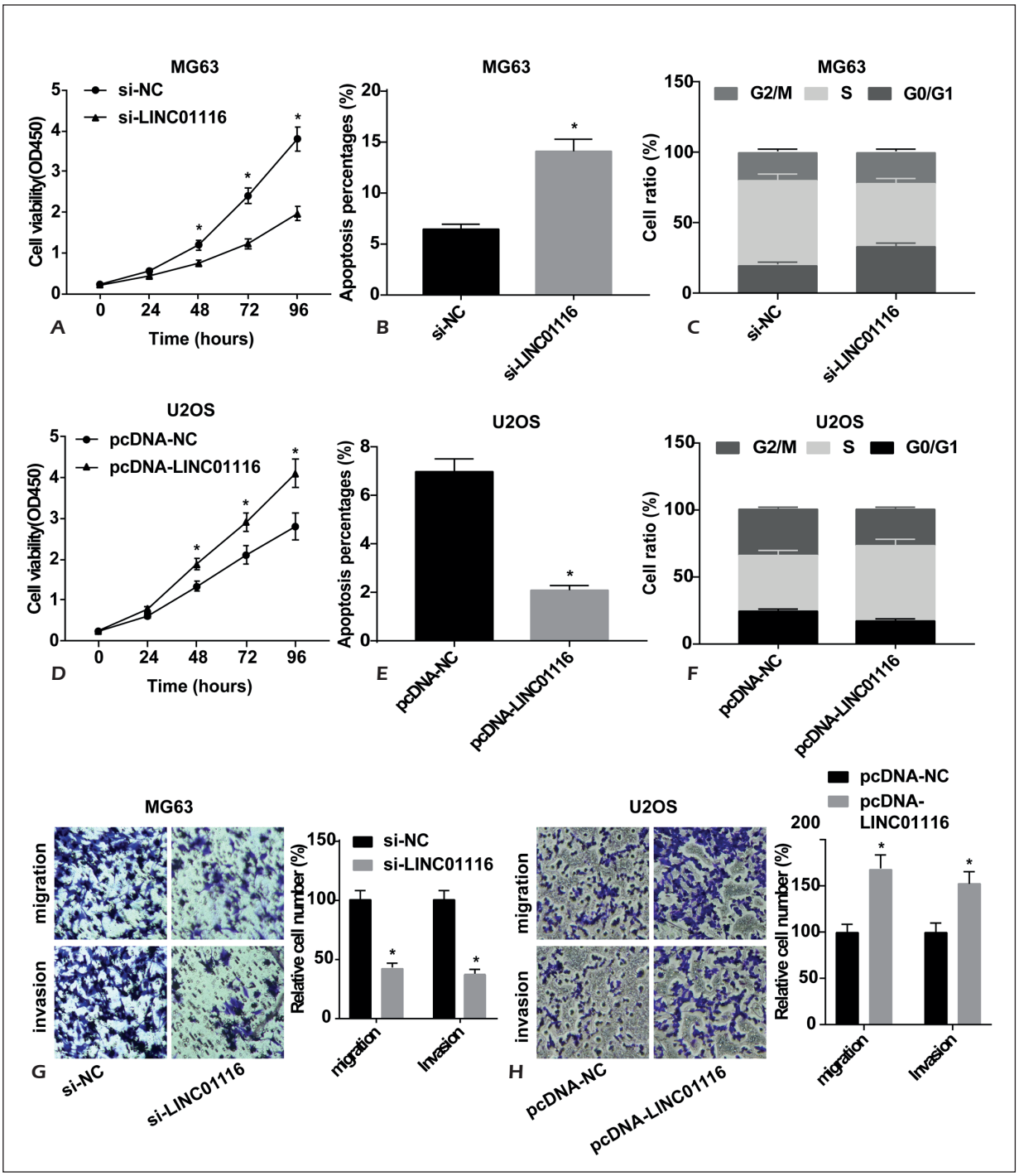

Figure 3. Overexpression of LINC01116 accelerated OS cells to proliferation, invasion and migration. ***A***, CCK-8 assay indicated that the viability of MG63 cells transfected with si-LINC01116 at 24, 48, 72, and 96 h was markedly reduced relative to that of controls. ***B***, Transfection of si-LINC01116 induced apoptosis of MG63 cells. ***C***, G0/G1 phase prolonged and S phase shortened in MG63 cells with LINC01116 knockdown relative to those of controls. ***D***, CCK-8 assay indicated that the viability of U2OS cells transfected with pcDNA-LINC01116 at 24, 48, 72, and 96 h was markedly elevated relative to controls. ***E***, Transfection of pcDNA-LINC01116 inhibited apoptosis of U2OS cells. ***F***, G0/G1 phase shortened and S phase prolonged in U2OS cells with LINC01116 overexpression relative to those of controls. ***G***, Migratory and invasive rates were significantly inhibited in MG63 cells transfected with si-LINC01116. H, Migratory and invasive rates were significantly elevated in U2OS cells transfected with pcDNA-LINC01116.

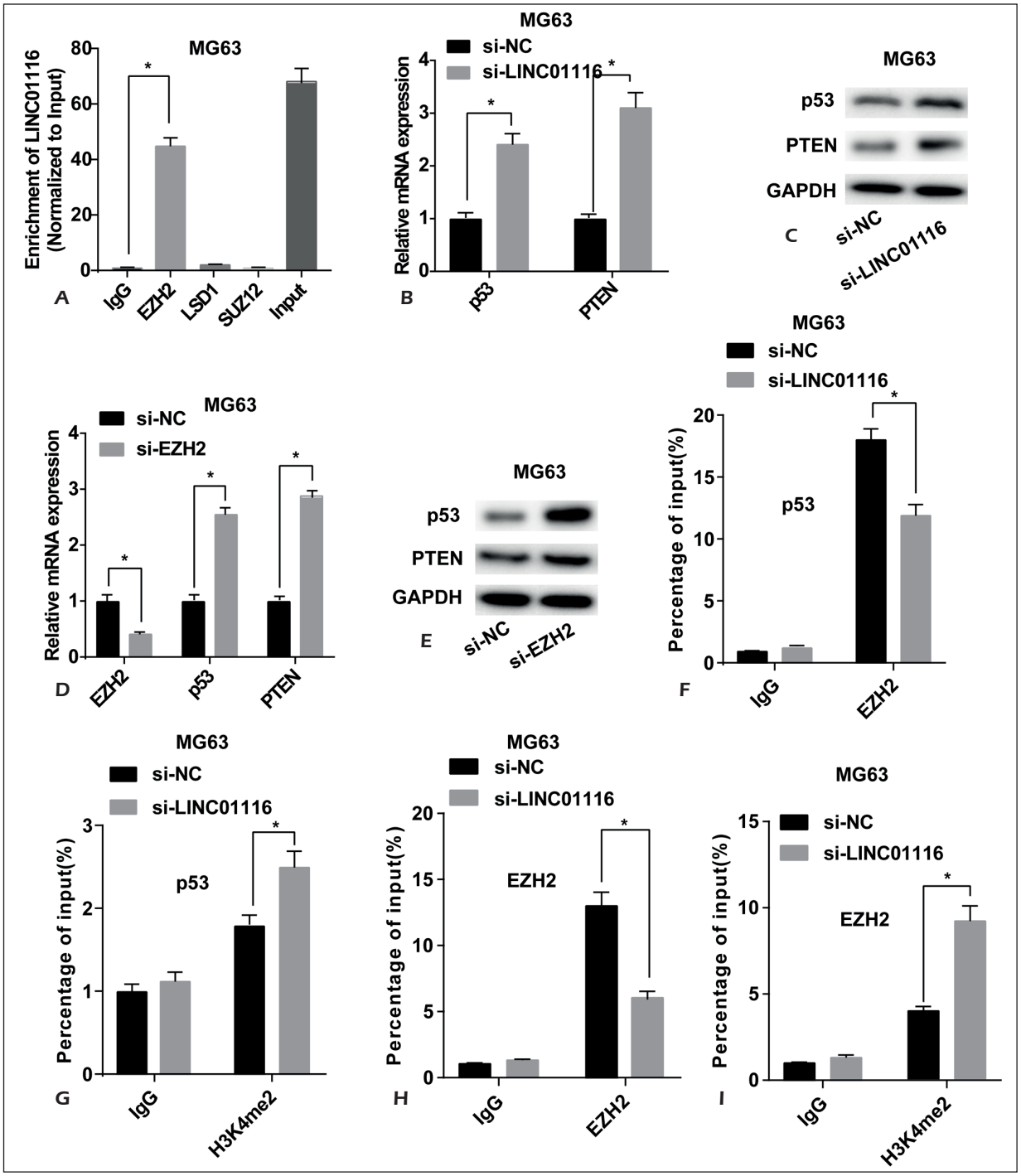

Figure 4. LINC01116 silenced p53 and PTEN by binding to EZH2. ***A***, LINC01116 was directly integrated with EZH2 rather than LSD1 and SUZ12. ***B-C***, Expressions of p53 and PTEN were negatively regulated by LINC01116 in MG63 cells at both mRNA (**B**) and protein levels (**C**). ***D-E***, Transfection of si-EZH2 in MG63 cells upregulated the mRNA (***D***) and protein levels (***E***) of p53 and PTEN. ***F***, LINC01116 knockdown in MG63 cells decreased the binding capacity of p53 to EZH2. ***G***, LINC01116 knockdown in MG63 cells promoted demethylation modification of H3K4me2 in p53. ***H***, LINC01116 knockdown in MG63 cells decreased the binding capacity of PTEN to EZH2. **I**, LINC01116 knockdown in MG63 cells promoted demethylation modification of H3K4me2 in PTEN.

of si-LINC01116 and si-EZH2 (Figure 5B). G0/G1 phase prolonged in MG63 cells with LINC01116 knockdown, while it was shortened by silencing EZH2 (Figure 5C). Identically, inhibited migratory and invasive abilities of MG63 cells resulted from LINC01116 knockdown were partially reversed by EZH2 knockdown (Figure 5D). The above findings concluded that LINC01116 promoted the proliferation, migration and invasion of OS cells, but inhibited cell apoptosis through down-regulating EZH2.

Discussion

OS is a primary bone malignancy, which mainly affects children and adolescents. It is highly invasive in the first stage of diagnosis and easily metastasizes to lungs. Significant advances have been achieved in therapeutic strategies, such as radiotherapy, adjuvant chemotherapy and extensive tumor resection. However, the prognosis of recurrent or metastatic OS is unsatisfactory. Therefore, it is necessary to determine the molecular mechanisms of OS, so as to identify specific biomarkers and therapeutic targets for OS. Recently Zhao H. et al[13] studies have identified the involvement of lncRNAs in the development and progression of various cancers, including breast cancer, gastric cancer, pancreatic cancer and OS. Several lncRNAs have been reported to regulate the progression of OS. For example, lncRNA HNF1A-AS1 is overexpressed in OS, which accelerates cell proliferation by activating Wnt/β-catenin pathway[14]. LncRNA FGFR3-AS1 promotes OS progression by activating FGFR3[15]. LINC01116 serves as an oncogene in breast cancer, ovarian

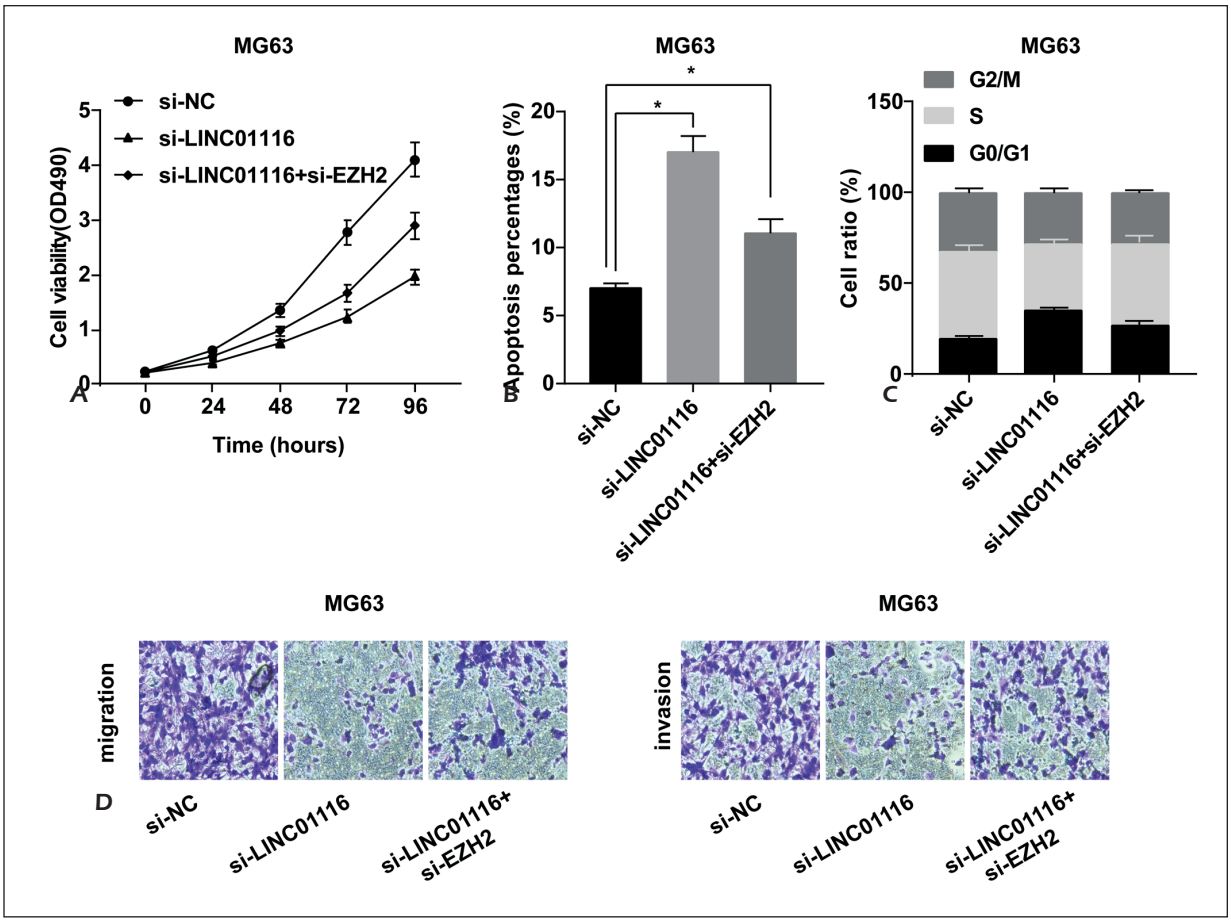

Figure 5. LINC01116 exerted its role in regulating OS cells through downregulating EZH2. MG63 cells were transfected with si-NC, si-LINC01116 or si-LINC01116+si-EZH2, respectively. ***A***, CCK-8 assay revealed that inhibited viability of MG63 cells caused by LINC01116 knockdown was partially reversed by EZH2 knockdown. ***B***, Induced apoptosis of MG63 cells resulted from LINC01116 knockdown was partially reversed by EZH2 knockdown. ***C***, Prolonged G0/G1 phase and shortened S phase in MG63 cells due to LINC01116 knockdown were partially reversed by EZH2 knockdown. ***D***, Inhibited migration and invasion of MG63 cells resulted from LINC01116 knockdown were partially reversed by EZH2 knockdown.

cancer and non-small cell lung cancer[16-18]. In our work, LINC01116 was highly expressed in OS cell lines and tissues. LINC01116 expression was negatively correlated with the overall survival of OS patients, which was consistent with previous researches. In addition, LINC01116 over-expression significantly promoted cell proliferative, invasive, migratory abilities and cell cycle progression, whereas inhibited cell apoptosis. Knockdown of LINC01116, conversely, yielded the opposite results. Our findings showed that LINC01116 promoted OS progression and could be considered as a promising target for OS treatment. As a catalytic subunit of polycomb repressive complex 2 (PRC2), enhancer of zeste homolog 2 (EZH2) catalyzes the trimethylation of H3K27 and exerts the function of transcriptional inhibition[19]. H3K27 trimethylation modifies chromatin conformation densification, leading to the silence of tumor-suppressor genes. This may eventually promote the progression of OS. Metastatic rate to lungs at the advanced stage of OS markedly decreases after EZH2 knockout. Meanwhile, cisplatin-sensitivity of OS cells increases. Moreover, EZH2 accelerates the proliferation and migration of OS cells, while inhibits cell apoptosis through down-regulating TSSC3[20]. P53 is a transcription factor that regulates key genes in DNA damage response, cell cycle progression and apoptotic pathways. P53 is a tumor-suppressor gene of all types of tumors, whose function may be affected by mutations of itself and upstream or downstream genes[21]. TP53 mutations with loss of function occur in 3/4 of OS cases, including allele loss, rearrangement, and point mutations[22-24]. A recent study has shown that rare exon mutations of TP53 or Li-Fraumeni syndrome are observed in 9.5% of young patients with osteosarcoma satellite lesions. However, these mutations disappear after OS onset[25]. PTEN is another important tumor-suppressor gene discovered after p53, which is closely related to various tumors[26]. In this study, LINC01116 regulated the transcriptional levels of p53 and PTEN by binding to EZH2. Silence of EZH2 partially reversed the functional effect of LINC01116 on cellular behaviors of OS cells, thus participating in the occurrence of OS. There were some shortcomings in this experiment. We only focused on the two important tumor-suppressor genes PTEN and p53 as potential targets for LINC01116. Searching for other potential targets of LINC01116 by high-throughput sequencing after exogenous over-expression or silence of LINC01116 is still needed to improve our conclusions.

Conclusions

Up-regulated LINC01116 promotes cell proliferation, invasion and cell cycle progression, inhibiting apoptosis of OC cells. Furthermore, LINC01116 is involved in the development of OC by binding to EZH2 to regulate expressions of PTEN and p53.

Conflict of Interests

The authors declared no conflict of interest.

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