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TCF3-activated LINC00152 exerts oncogenic role in osteosarcoma through regulating miR-1182/CDK14 axis

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ABSTRACT

Long noncoding RNAs (lncRNAs) have been reported to participate in tumorigenesis and diverse cellular processes in osteosarcoma (OS). However, the role of lncRNA LINC00152 in OS remains elusive. In this study, LINC00152 was highly expressed in osteosarcoma tissues and cell lines. Moreover, MTT and colony formation assays revealed that knockdown of LINC00152 significantly suppressed cell proliferation. The inhibitory effect of LINC00152 knockdown on OS cell migration and invasion was analyzed and demonstrated by transwell assays. Additionally, Chromatin immunoprecipitation (ChIP) and luciferase reporter assays suggested that LINC00152 was transcriptionally activated by the transcription factor TCF3. More importantly, mechanism investigation revealed that LINC00152 was predominantly located in the cytoplasm of OS cells and acted as a competing endogenous RNA (ceRNA) in OS by regulating miR-1182/CDK14 axis. Collectively, LINC00152 was activated by TCF3 and promotes cell proliferation and migration in osteosarcoma via miR-1182-CDK14 axis.

1. Introduction

Osteosarcoma (OS) is a common bone tumor that often prevail in childhood [9,23]. Moreover, osteosarcoma originates from bone-forming mesenchymal cell, characterized by highly invasive and distant metastasis [26,31]. Although current progression in radiotherapy and chemotherapy have dramatically promoted the survival rate of osteosarcoma patients, the prognosis of patients with distant metastasis is still poor [12,33]. Reports suggested that the 5-year survival rate of OS patients with non-distant metastasis was approximately 60%–70%. However, 5-year survival rate of osteosarcoma patients with distant metastasis is only 20–30% [2,24]. The function of genetic abnormalities in osteosarcoma has been reported [25,28]. However, the pathogenesis of osteosarcoma remains largely unclear. Therefore, detecting the molecular mechanism of osteosarcoma is significant for finding more novel therapeutic targets.

Increasing studies suggested the regulatory role of lncRNAs in tumor initiation and development [17,22,27]. Moreover, lncRNAs were reported to be an oncogene or tumor inhibitor in various cancers [5,19,36]. It has been reported that LINC00152 was highly expressed in several cancers and associated with tumor growth and metastasis [4,6,15,16]. Moreover, abnormal expression of LINC00152 could regulate cancer occurrence and progression [35]. However, the expression pattern and function of LINC00152 in osteosarcoma are almost unknown. Therefore, the aim of this study is to investigate the role of LINC00152 in OS. At first, the expression pattern of LINC00152 was identified in OS tissues and cell lines. The correlation between LINC00152 expression and the clinicopathological features and the overall survival of OS patients was analyzed. Loss-of function assays were carried out to determine the effect of LINC00152 knockdown on the cellular processes in OS, such as proliferation, invasion and migration. Mechanistically, the upstream and downstream molecular mechanism of LINC00152 was analyzed using bioinformatics analysis and mechanism experiments. Finally, rescue assays were designed and carried out. In summary, this study revealed the mechanism and function of LINC00152 in osteosarcoma progression.

2. Material and methods

2.1. Tissue samples

84 pairs of osteosarcoma tissues and adjacent normal tissues (the distance from tumor tissues > 5 cm) were received from osteosarcoma patients at The Second Affiliated Hospital of Suzhou University.

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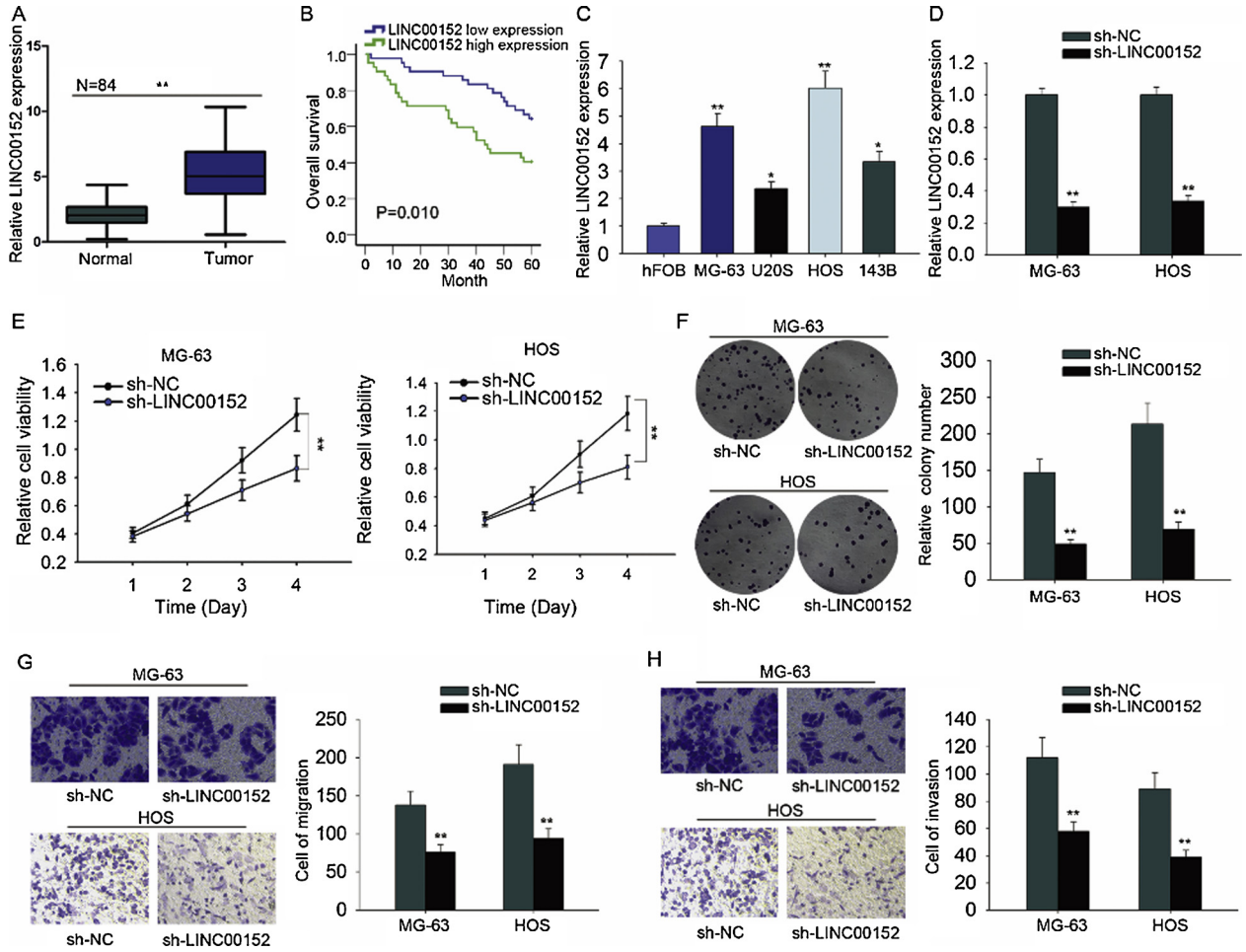

Fig. 1. Knockdown of LINC00152 inhibited cell proliferation and invasion in OS. (A) qRT-PCR assay showed the expression level of LINC00152 in 84 pairs of OS tissues and adjacent normal tissues. (B) The association between the expression of LINC00152 and the overall survival of OS patients was analyzed. (C) The expression level of LINC00152 in four OS cell lines compared with that in the normal cell line. (D) The expression level of LINC00152 in MG-63 and HOS cells transfected with sh-NC or sh-LINC00152. (E–F) Cell proliferation of MG-63 and HOS cells transfected with sh-NC or sh-LINC00152 was measured by MTT and colony formation assays. (G–H) The migration and invasion of MG-63 and HOS cells transfected with sh-NC or sh-LINC00152 by transwell assays. $^{*}p < 0.05$, $^{**}p < 0.01$.

Patients without preoperative treatments (radiotherapy or chemotherapy) were eligible for our study. Informed consent was obtained from all osteosarcoma patients prior to operation. Instantly after surgical resection, tissue specimens were frozen and stored in liquid nitrogen at -80 °C before using. This study is approved by the ethics committee of The Second Affiliated Hospital of Suzhou University.

2.2. Cell culture and transfection

Four osteosarcoma cell lines (U20S, MG-63, HOS, 143B) and one normal human fetal osteoblastic cell line (hFOB) were obtained from American Type Culture Collection (ATCC, Rockville, Maryland, USA) and Type Culture Collection Center (Shanghai, China), respectively. Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Life Technologies, Carlsbad, CA, USA) together with 10% FBS (Gibco BRL, Grand Island, NY, USA), 100 U/ml penicillin as well as 100 μg/ml streptomycin. Cell lines were incubated in humid atmosphere consisting of 5% CO_2. Plasmid transfection was conducted in MG-63 and HOS cells using Lipofectamine2000 (Invitrogen, USA). To knock down LINC00152 expression, the short hairpin RNA (shRNA) against LINC00152 (sh-LINC00152, Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) was designed. A negative control (sh-NC) was obtained from RiboBio (Guangzhou, China). The sequence of sh-LINC00152 was: AAGGTTACTCTTACTGACAGTTCATTTT. The sequence of sh-NC was: CGATGCCAGACGCACAGACTCGTCCCGG. MiR-1182 mimics/inhibitor and pcDNA-CDK14 as well as their negative controls were bought from GenePhama (Shanghai, China).

2.3. RNA isolation and qRT-PCR

Total RNA was extracted from osteosarcoma tissues and cell lines by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's protocols. The concentration and purity of total RNA were assessed using spectrophotometric method (Bio-Rad, Hercules, CA, USA). Reverse transcription was conducted using the iScript cDNA Synthesis Kit (Bio-Rad). The quantitative real-time PCR (qRT-PCR) was performed using SYBR Premix EX Taq™ Kit (Takara, Dalian, China) and ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, USA) in line with the standard methods. GAPDH and U6 were used as the internal references. The genes expression was analyzed by using $2^{-\Delta\Delta Ct}$ method.

2.4. MTT analysis

Cell viability was detected using methylthiazoletetrazolium (MTT) assay. MG-63 or HOS cells (4×10^3/per well) were incubated in 96-well plates (Sigma-Aldrich, St. Louis, MO, USA). After incubation at 37 °C with 5% CO_2 for 24 h, 10 μl of MTT solution (5 mg/ml) was added to each well at different time points (24, 48, 72, 96 h). 150 μl DMSO (Sigma-Aldrich) was applied to solubilize the MTT crystals. Then, the

Table 1
Correlation between the expression of LINC00152 and clinicopathological features of patients with osteosarcoma. (n = 84).

Variable	LINC00152 expression		P-value
	Low	High	
Age			
< 60	15	17	0.822
≥60	27	25	
Gender			
Male	25	22	0.660
Female	17	20	
Tumor Size	22	11	0.024*
< 3	20	31	
≥3			
Location			
Tibia/femur	28	25	0.651
Elsewhere	14	17	0.655
Differentiated Degree			0.023*
High/middle	15	18	0.826
Low/undifferentiation	27	24	0.028*
Clinical Stage			
I	21	10	
II	21	32	
Histological Type	18	20	
Osteoblastoma	24	22	
Else	26	15	
TNM Stage	16	27	
I-II			
III-IV			

Low/high decided by the sample mean. Pearson χ^2 test. *P < 0.05 was considered statistically significant.

absorbance of plate at 570 nm was analyzed by microplate reader (Thermo Scientific, Rockford, Illinois, USA).

2.5. Colony formation assay

The transfected MG-63 or HOS cells (3×10^2/per well) were cultured in 6-well plates and cultured in DMEM with 10% FBS at 37 °C with 5% CO_2. Culture medium was replaced every third day. After two weeks, colonies were washed three times in phosphate-buffered saline (PBS, Invitrogen, CA, USA) and fixed with 10% formaldehyde for half an hour. Next, the fixed cells were stained with 0.1% crystal violet (Sigma-Aldrich) in PBS for 15 min. The number of colony cells was calculated manually.

2.6. Transwell assays

For invasion or migration assay, the Transwell chambers (8 mm pore filter; Corning Company, New York, USA) were coated with or without Matrigel (BD Biosciences, San Jose, CA, USA). 48 h post-transfection, MG-63 and HOS cell lines were rinsed twice in PBS solution. Cells were suspended in serum-free DMEM medium with 10% FBS. Cell concentration was adjusted to 2×10^6 cells/mL. Thereafter, 200 μL cell suspension in serum-free medium were added to the upper chamber. 500 μL of complete DMEM medium supplemented with 10% FBS was added to the lower chamber. After 24 h, migrated and invaded cells were fixed with methanol for 30 min and stained with crystal violet solution for 1 h. Cells on the upper chamber were removed using the cotton swab. The stained cells were counted in 5 random fields using a CKX41 inverted microscope (Olympus Corporation, Tokyo, Japan).

2.7. Subcellular fractionation

The localization of LINC00152 in cytoplasm or nucleus was measured using Cytoplasmic and Nuclear RNA Purification Kit (Norgen, Thorold, ON, Canada). 1×10^7 MG-63 or HOS cells were cultured in 500 μl cytoplasm lysis buffer on ice for three minutes. The cytoplasm supernatant was placed into a clean tube. Nuclear fractions were re-suspended in 100 μl nucleus lysis buffer at 4 °C for one hour. After centrifugation, nuclear fractions were extracted. Then, qRT-PCR was utilized to detect the expression level of LINC00152 in cytoplasm and nucleus. GAPDH was regarded as the cytoplasmic control, whereas U6 was utilized as the nuclear control.

2.8. Dual-luciferase reporter analysis

The 3′-UTR of LINC00152 was amplified and sub-cloned into downstream of firefly luciferase report plasmid in pmirGLO reporter vector (Promega, Madison, WI, USA). The wild-type of LINC00152 (LINC00152-WT) was generated. The GeneTailor™ Site-Directed Mutagenesis System (Invitrogen, USA) was applied to construct mutant type of LINC00152 (LINC00152-MUT). The pmirGLO-LINC00152 or pmirGLO-LINC00152-MUT was co-transfected with miR-1182 mimics and miR-NC into MG-63 or HOS cells. 48 h later, the luciferase activity was detected using Dual-luciferase Reporter Assay System (Promega) following the user guide.

2.9. RIP assay

RIP assay was performed using Magna RIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA). Briefly, MG-63 and HOS cell lines were lysed in RNA lysis buffer. Cell lysates were cultured in RNA immunoprecipitation buffer, which contained magnetic beads conjugated with human Argonaute 2 (Ago2) antibody (Millipore) or negative control mouse IgG antibody (Millipore). Subsequently, these samples were digested with proteinase K. The immunoprecipitated RNA complex was isolated. Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and the bio-analyzer (Agilent, Santa Clara, CA, USA) were used to measure the concentration and quality of RNA. Moreover, purified RNAs were detected using quantitative real-time PCR.

2.10. Northern blot analysis

30 μg of total cellular RNA was isolated from MG-63 and HOS cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and purified using RNAeasy (Qiagen, Valencia, CA). Next, RNA was quantitated by means of spectrophotometer, followed by preservation at -80 °C. Total RNA specimen was size-fractionated by electrophoresis on 17% PAGE gels (BioRad Laboratories, Hercules, CA, USA) and electrically transferred onto 0.45 mm of Hybond-N + nitrocellulose membranes (Amersham, Freiburg, Germany). After drying at 80 °C and cross-linking under ultraviolet light, the blots were hybridized with biotinylated oligonucleotide probes (Roche, Basel, Switzerland) specific to LINC00152 in hybridization buffer (Toyobo, Shanghai, China) at 50 °C overnight in accordance with the user manual provided by supplier. Thereafter, the blots were rinsed three times for half an hour and blotted dry. At length, the blots were instantly exposed to a Phosphor Imager screen (SigmaAldrich, St. Louis, MO, USA) at -80 °C for ten hours. The signal strength of hybridization bands was quantified by densitometry using Image Quant software (Amersham, Germany).

2.11. Western blot analysis

MG-63 and HOS cell lines were placed into 6-well plates and lysed with RIPA buffer (Beyotime Institute of Biotechnology, Guangzhou, China) on ice. BCA protein assay kit (Beyotime Institute of Biotechnology, China) was used to analyze the protein concentration. All samples were separately subjected to SDS-PAGE (Thermo Scientific, Rockford, Illinois, USA) and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). Subsequently, membranes were blocked in 5% nonfat dry milk for 1 h and then incubated with primary

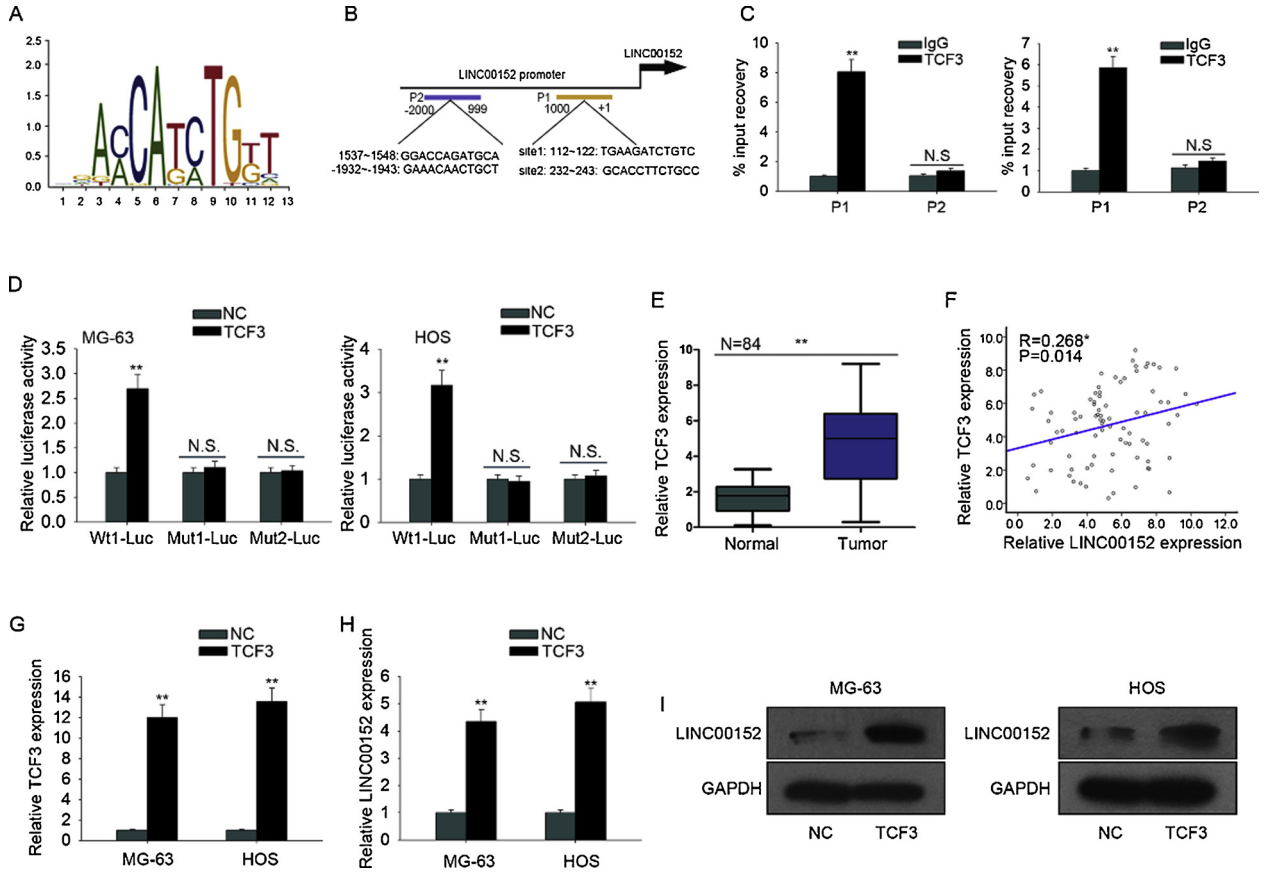

Fig. 2. LINC00152 is activated by TCF3 in OS. (A) The binding motif of transcription factor TCF3. (B) TCF3 response elements in LINC00152 promoter region. (C) ChIP assay demonstrated the binding of TCF3 to the part 1 of (P1) LINC00152 promoter region. (D) The interaction between TCF3 and site 1 or site 2 of LINC00152 promoter was determined by luciferase reporter assay. (E) The expression level of TCF3 was analyzed in OS tissues and adjacent normal tissues. (F) Pearson correlation analysis showed the correlation between TCF3 and LINC00152 in OS tissues. (G) The level of TCF3 was increased in MG-63 and HOS cells transfected with NC and pcDNA-TCF3. (H–I) The expression level of LINC00152 was measured in MG-63 and HOS cells transfected with NC and pcDNA-TCF3 by qRT-PCR and northern blot assay. $^{*}p < 0.05$, $^{**}p < 0.01$. N.S: no significance.

antibodies against CDK14 (#DCABH-201246, Creative Diagnostics, New York, NY, USA) and GAPDH (#ab8245, Abcam, Cambridge, Massachusetts, USA) at 4 °C overnight. The secondary antibody conjugated horseradish peroxidase was incubated with membranes for 2 h. GAPDH was used as an internal control. Protein bands were visualized with enhanced chemiluminescence (ECL) kit (Santa Cruz Biotechnology).

2.12. Statistical analysis

Statistical analysis was analyzed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation (SD). Differences were analyzed using Student's *t*-test and one-way analysis of variance (ANOVA) with the Tukey post hoc test. Pearson correlation analysis was performed to measure the expression relations among LINC00152, miR-1182 and CDK14. Each experiment was conducted at least three times. Statistical significance was presented as p value less than 0.05.

3. Results

3.1. Knockdown of LINC00152 inhibited cell proliferation, migration and invasion in OS

At first, we detected the expression of LINC0052 in OS. qRT-PCR analysis revealed that LINC00152 was overexpressed in osteosarcoma tissues compared with adjacent normal tissues (Fig. 1A). Using the mean expression level of LINC00152 as the threshold, we classified 84 OS samples into two groups: LINC00152 high expression group and LINC00152 low expression group. Next, we analyzed the correlation between LINC00152 expression and the clinicopathological features of OS patients. As shown in Table 1, the expression of LINC00152 had close associations with tumor size. TNM stage and clinical stage. Kaplan-Meier method revealed that patients with high LINC00152 expression had a relative lower overall survival rate that those with low LINC00152 expression (Fig. 1B). Similarly, we detected the relative high expression level of LINC00152 in OS cell lines, especially in MG-63 and HOS cell lines (Fig. 1C). Therefore, LINC00152 was downregulated in MG-63 and HOS cells by transfecting with sh-LINC00152 (Fig. 1D). To detect the effect of LINC00152 on biological processes of OS cells, functional assays were performed. MTT and colony formation assays suggested that knockdown of LINC00152 remarkably suppressed cell proliferation in both MG-63 and HOS cell lines (Fig. 1**E–F**). In addition, transwell assays showed that the migration and invasion of MG-63 and HOS cells were efficiently inhibited by transfecting sh-LINC00152 (Fig. 1**G–H**).

3.2. LINC00152 is activated by TCF3 in OS

To investigate the mechanisms which contributed to the upregulation of LINC00152 in OS, bioinformatics online tools were utilized. According to the search results of UCSC (http://genome.ucsc.edu/) and JASPAR (http://jaspar.genereg.net/), TCF3 is a potential transcription factor for LINC00152. The binding motif of TCF3 was obtained

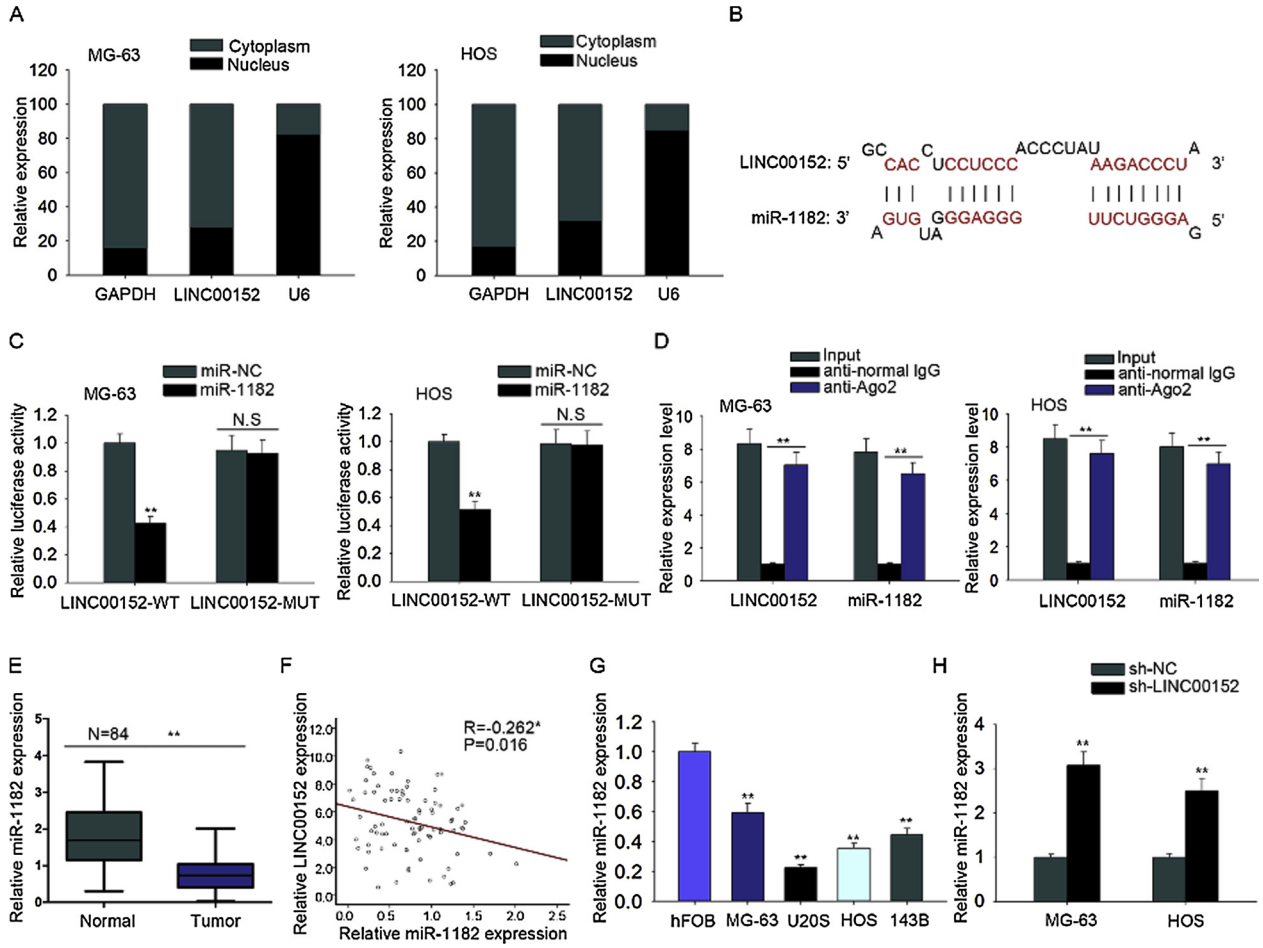

Fig. 3. LINC00152 acted as a molecular sponge of miR-1182 in OS. (A) Subcellular fractionation assay revealed the localization of LINC00152. (B) The binding sequence between LINC00152 and miR-1182 was obtained. (C) Luciferase reporter assay showed the luciferase activity of wild type LINC00152 (LINC00152-WT) and mutant type LINC00152 (LINC00152-MUT) in MG-63 and HOS cells transfected with miR-NC or miR-1182 mimics. (D) RIP assay further detected the interaction between LINC00152 and miR-1182 in OS cells. (E) The expression of miR-1182 was assessed in OS tissues and corresponding normal tissues using qRT-PCR assay. (F) The expression correlation between LINC00152 and miR-1182 was tested using Pearson correlation analysis. (G) The expression of miR-1182 in OS cell lines and one normal cell line was measured by qRT-PCR. (H) The effect of LINC00152 knockdown on miR-1182 was measured using qRT-PCR assay. $^{*}p < 0.05$, $^{**}p < 0.01$. N.S: no significance.

(Fig. 2A). We then selected the top four binding sequences for further analysis (Fig. 2B). Then, ChIP assays revealed the affinity of TCF3 to part 1 (P1) of LINC00152 promoter (Fig. 2C). To make further confirmation, we employed luciferase reporter assay. Results revealed that TCF3 increased the luciferase activity of both site 1 and site 2 of P1 fragment (Fig. 2D). However, TCF3 could not affect the luciferase activity after site 1 and site 2 were mutated. In addition, we found that TCF3 was upregulated in OS tissues (Fig. 2E) and positively correlated with that of LINC00152 (Fig. 2F). To detect the effect of TCF3 on the LINC00152 expression, TCF3 was overexpressed in both MG-63 and HOS cells (Fig. 2G). Results demonstrated that overexpression of TCF3 dramatically increased the level of TCF3 in MG-63 and HOS cells. Furthermore, the results of qRT-PCR and northern blot assays showed that TCF3 overexpression significantly increased the expression levels of LINC00152 in MG-63 and HOS cells (Fig. 2**H–I**).

3.3. LINC00152 acted as a molecular sponge of miR-1182 in OS

In this study, we found that LINC00152 was enriched in the cytoplasm of OS cells, indicating that LINC00152 might regulate its downstream gene expression at post-transcriptional level (Fig. 3A). Thus, we hypothesized that LINC00152 might act as a ceRNA in OS. Subsequently, we found out the miRNAs which potentially bind with LINC00152 by searching on DIANA (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php). Among which, miR-1182 has not been reported in OS and exhibited higher binding scores over 0.98. Therefore, we chose miR-1182 for further analysis. And the binding sites between LINC00152 and miR-1182 were illustrated (Fig. 3B). Then, luciferase reporter assay demonstrated that upregulation of miR-1182 notably decreased the luciferase activity of LINC00152-WT (wild-type), but had no effect on LINC00152-MUT (mutant-type) in MG-63 and HOS cells (Fig. 3C). RIP assay further confirmed that LINC00152 and miR-1182 were enriched in Ago2 pellet in MG-63 and HOS cells, suggesting that the binding of LINC00152 to miR-1182 (Fig. 3D). Subsequently, qRT-PCR assay revealed that miR-1182 was significantly downregulated in OS tissues (Fig. 3E). Furthermore, the expression of miR-1182 was negatively associated with that of LINC00152 (Fig. 3F). Besides, we also found that miR-1182 was significantly downregulated in OS cell lines (Fig. 3G). Importantly, knockdown of LINC00152 dramatically enhanced the level of miR-1182 in MG-63 and HOS cells (Fig. 3H).

3.4. LINC00152 upregulated CDK14 expression by competitively binding miR-1182

To investigate the underlying molecular mechanism of miR-1182 in osteosarcoma, we found out the target gene of miR-1182 by using TargetScan (http://www.targetscan.org/vert_72/). Among these

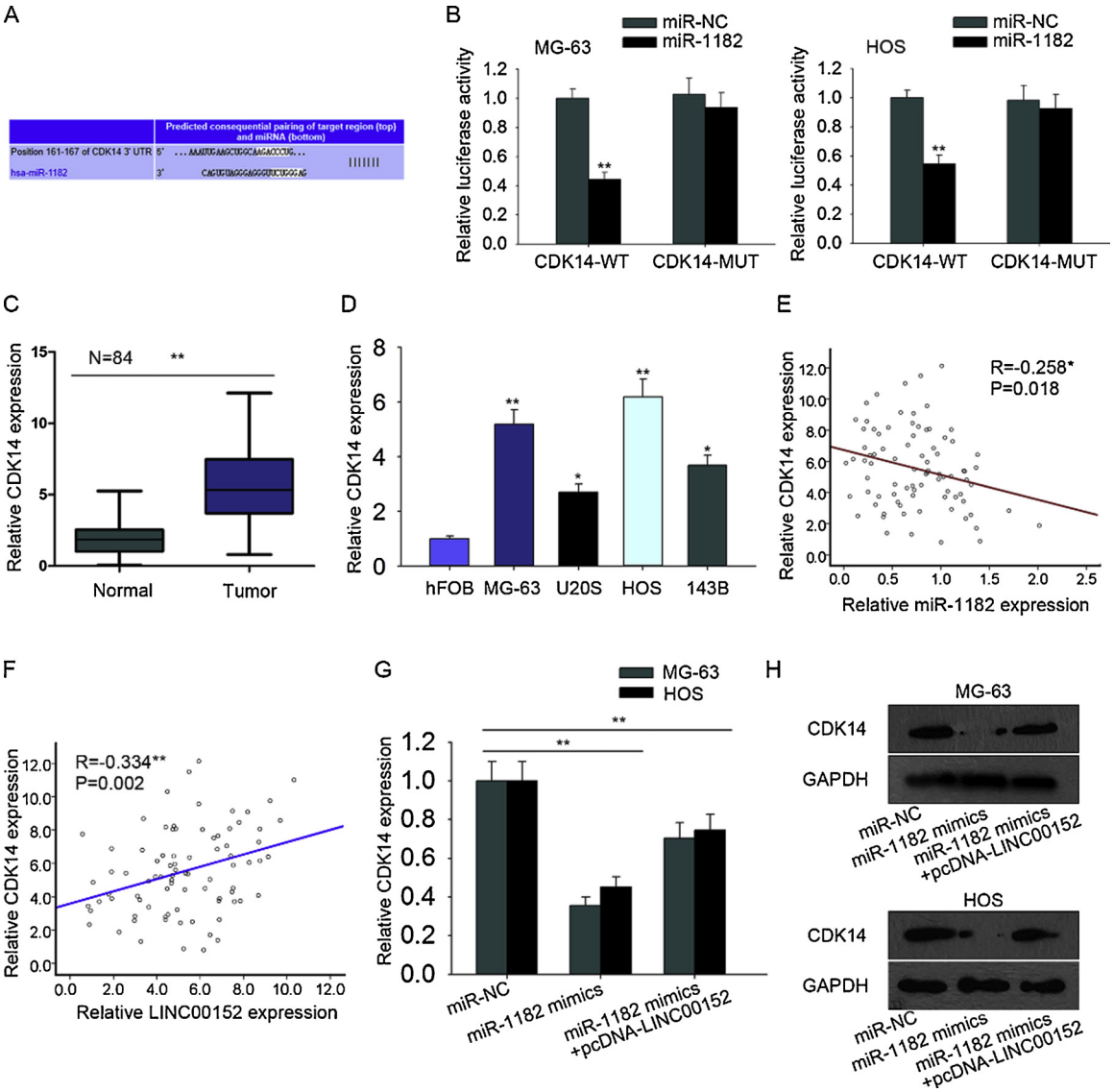

Fig. 4. LINC00152 upregulated CDK14 expression by competitively binding miR-1182. (A) The binding sites between miR-1182 and CDK14 was obtained from targetScan. (B) Dual luciferase reporter assay further demonstrated the combination between miR-1182 and CDK14 in MG-63 and HOS cells. (C–D) The expression of CDK14 in OS tissues and cell lines was analyzed by qRT-PCR assay. (E–F) The expression correlation between CDK14 and miR-1182 as well as between CDK14 and LINC00152 were measured using Pearson correlation analysis. (G–H) qRT-PCR and western blot assays manifested the effect of miR-1182 upregulation or LINC00152 overexpression on the mRNA and protein levels of CDK14. $^{*}p < 0.05$, $^{**}p < 0.01$.

mRNAs, CDK14 has been reported to participate in ceRNA network in osteosarcoma [7,10]. Therefore, we chose CDK14 to do further mechanism investigation. Then, the binding sites between miR-1182 and CDK14 were obtained from TargetScan (Fig. 4A). In addition, luciferase reporter assay confirmed that miR-1182 mimics significantly decreased the luciferase activity of wild type CDK14 (CDK14-WT), while no change was observed in mutant type CDK14 (CDK14-MUT) (Fig. 4B). Subsequently, the expression level of CDK14 was found to be much higher in OS tissues and cell lines compared with that in normal tissues or cell lines (Fig. 4**C–D**). Furthermore, the negative expression association between CDK14 and miR-1182 as well as between CDK14 and LINC00152 were analyzed by Pearson correlation analysis (Fig. 4**E–F**). Additionally, we found that miR-1182 mimics obviously reduced the mRNA level and protein level of CDK14, while the results were reversed by co-transfection with pcDNA-LINC00152 (Fig. 4**G–H**). In summary, these findings elucidate that LINC00152 competitively bind with miR-1182 to elevate CDK14 expression in osteosarcoma.

3.5. LINC00152 promoted cell proliferation, migration and invasion in osteosarcoma through regulating miR-1182/CDK14 axis

Finally, we conducted rescue assays to confirm the function of LINC00152-miR-1182-CDK14 axis in OS cell proliferation, migration and invasion. It was found that cell proliferation induced by LINC00152 knockdown was partly reversed by miR-1182 inhibitor or pcDNA-CDK14 (Fig. 5**A–B**). Additionally, the inhibitory effects of LINC00152 knockdown on cell migration and invasion were partially rescued by miR-1182 inhibitor or pcDNA-CDK14 (Fig. 5**C–D**). These results suggested that LINC00152 promoted osteosarcoma progression via modulating miR-1182/CDK14 axis.

4. Discussion

Recently, increasing literature revealed the important role of lncRNAs in human cancers, including osteosarcoma [11,18]. The abnormal expression of lncRNAs was closely associated with the tumor growth and metastasis [1]. LncRNA LINC00152 has been reported to be

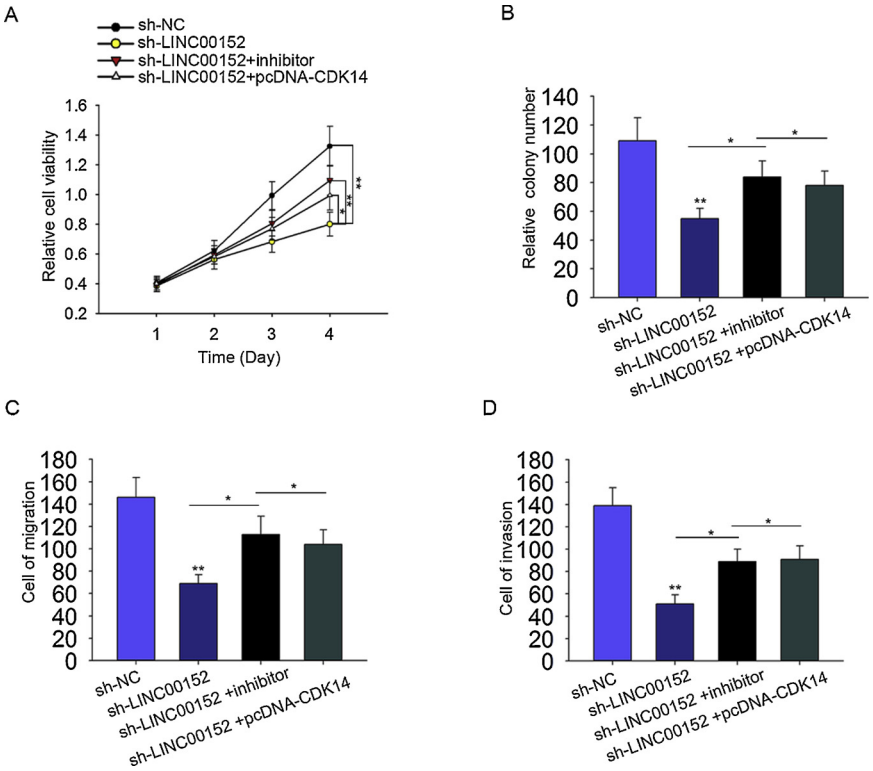

Fig. 5. LINC00152 promoted cell proliferation, migration and invasion in osteosarcoma through regulating miR-1182/CDK14 axis. (A–B) The effect of miR-1182 inhibitor or pcDNA-CDK14 on sh-LINC00152-mediated cell proliferation were examined using MTT and colony formation assays. (C–D) Cell migration and invasion were evaluated in OS cells co-transfected with sh-LINC00152 and miR-1182 inhibitor or in cells co-transfected with sh-LINC00152 and pcDNA-CDK14. $^{*}p < 0.05$, $^{**}p < 0.01$.

highly expressed in various cancers, such as lung cancer, hepatocellular cancer and renal cell carcinoma. It is unknown that whether LINC00152 was correlated with the OS progression. Therefore, we investigated the function of LINC00152 in OS. At first, the expression level of LINC00152 was found to be higher in both OS tissues and cell lines compared with that in corresponding normal controls. Furthermore, the clinical potential of LINC00152 was analyzed. Interestingly, the expression of LINC00152 was negatively correlated with the overall survival rate of OS patients, indicating the potential involvement of LINC00152 in OS progression. Moreover, the expression of LINC00152 was associated with tumor size, TNM stage and clinical stage, indicting the potential association of LINC00152 with the clinical features of OS patients. Functionally, dysregulation of lncRNAs is associated with the abnormal biological processes in human cancers [13,29,34]. Therefore, we applied loss-of function assays to detect the effect of LINC00152 on OS cell proliferation, migration and invasion. Unsurprisingly, knockdown of LINC00152 dramatically suppressed osteosarcoma cell proliferation, invasion and migration. These results indicated that LINC00152 exerted pro-oncogenic function in osteosarcoma progression.

Previous studies showed that lncRNAs can be transcriptionally activated by various transcription factors [8,32]. Thus, we investigated whether LINC00152 upregulation was induced by its upstream transcription factors. Based on bioinformatics analysis, we found that TCF3 was the potential transcription factor of LINC00152. Then. We analyzed the interaction between TCF3 and LINC00152 promoter by using ChIP assay and luciferase reporter assay. In addition, TCF3 positively regulated the expression of LINC00152. The results suggested that TCF3 could activate the LINC00152 transcription. Thus, we confirmed that LINC00152 was activated by TCF3 and promoted OS cell proliferation, migration and invasion.

Recently, reports demonstrated that lncRNAs can increase the expression levels of mRNAs by sponging miRNAs [3,20]. Previously, LINC00152 has been reported to participate in ceRNA pathway by regulating miRNA-mRNA axis [21,30]. Since we found that LINC00152 was located in the cytoplasm of OS cells, we hypothesized that LINC00152 might act as a ceRNA in OS. Using bioinformatics analysis, we predicted the potential interaction between miR-1182 and LINC00152. Previous reports documented that miR-1182 has been certified as tumor inhibitor in ovarian cancer and bladder cancer [14,37]. In the current study, mechanism experiments, including dual-luciferase reporter assay and RIP assay, proved that LINC00152 could bind with miR-1182 in OS cells. The negative regulation of LINC00152 on miR-1182 was analyzed in OS cells. All these findings demonstrated that LINC00152 was a molecular sponge of miR-1182 in OS cells. Likewise, the target mRNA of miR-1182 was found out and verified. All these experimental results suggested that LINC00152 acted as a ceRNA by sponging miR-1182 to upregulate CDK14 expression in OS. Finally, rescue assays confirmed that miR-1182 and CDK14 involved in LINC00152-mediated OS cell proliferation, invasion and migration, indicating the function of LINC00152-miR-1182-CDK14 axis in OS. In conclusion, all experimental results in this study revealed that LINC00152 is activated by TCF3 and exerts oncogenic role in OS through regulating miR-1182/CDK14 axis. Our research findings may contribute to the study on the novel therapeutic target for OS.

Conflict of interest

There are no conflicts of interest to disclose.

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Not applicable.

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