LncRNA NCRNA00173 is down-regulated in pediatric osteosarcoma and suppresses cell metastasis of osteosarcoma cells through regulating PI3k/Akt pathway

Zhongquan Zhao
Liaocheng Third People Hospital
Jiechao Chen (chenjiechao1680@163.com)
Liaocheng Dongchangfu District Maternal and Child Health Hospital
Dezhi Xia
Linyi People's Hospital

Research article

Keywords: pediatric osteosarcoma, LncRNA NCRNA00173, PI3K/Akt pathway, metastasis, Orthopedics

Posted Date: December 18th, 2019

DOI: https://doi.org/10.21203/rs.2.19195/v1

Abstract

Background: Osteosarcoma (OS) is a primary malignant tumor with high mortality and disability rate in childhood and adolescent, whereas the influence of LncRNA00173 (NCRNA00173) on pediatric OS progression is not obvious yet. Therefore, this study aimed to investigate the expression of NCRNA00173 in pediatric OS and its effect on OS progression.

Methods: In our study, qRT-PCR was adopted to test the NCRNA00173 expression in 40 pairs of pediatric OS tissues and OS cell lines. Kaplan-Meier method and Cox proportional hazard model were performed to analyze the prognosis of pediatric OS patients. The cell proliferation, apoptosis, migration and invasion of U2OS and HOS cells were test by MTT assay, flow cytometry, wound-healing, and transwell assay, respectively. The protein expression levels of PI3K/Akt pathway were measured by western blot. In addition, tumor growth in nude mice was also detected.

Results: The expression of NCRNA00173 was down-regulated and relevant with poor prognosis in pediatric OS. Overexpression of NCRNA00713 inhibited cell proliferation, migration and invasion, as well as accelerated cell apoptosis in U2OS and HOS cells. Overexpression of NCRNA00713 suppressed tumor growth in nude mice. The protein expression of p-PI3K and p-Akt were remarkedly decreased in U2OS and HOS cells after transfection with NCRNA00173. In addition, 740 Y-P (PI3K/Akt pathway activator) eliminated the impact of NCRNA00173 in HOS.

Conclusions: NCRNA00173 was down-regulated in pediatric OS and suppressed metastasis of OS cells by regulating PI3k/Akt pathway.

Background

Osteosarcoma (OS) is the most familiar primary bone tumor in adolescents, which occurs in the metaphysis of a long bone with rapid growth and has an annually incidence rate of about 6–8 per million. [1,2] It is characterized by metastasis and poor prognosis and mainly transferred through the blood and lungs. The survival rate of OS patient is only 28% within 5 years. [3] Despite survival rate of OS patient has increased to 60% within 5 years via excision, chemotherapy and radiotherapy, but they exist the highly chance of recurrence and distant metastasis. [4] Due to lack of early diagnostic indicators, some OS patients were found to be in advanced stage or to have lung metastasis and deficiency. [5] Therefore, it is necessary for us to further demonstrate pathogenesis in OS progression and find a biomarker to predict prognosis.

LncRNAs are a novel group of non-protein-coding transcript RNAs, with exceeding 200 nt in length and not involved in translation, [6] and play vital functions on OS progression. [7] For example, LncRNA HOXA11-AS is overexpressed and regulates miR-125a-5p/Rab3D in promoting OS metastasis. [8] Lian H et al. [9] have declared that Linc00460 could promote cell metastasis via building ceRNA and be functioned as

biomarker of prognosis in OS. LncRNA NCRNA00173 is down-regulated and acts as a novel molecular biomarker for HCC. [10] However, the expression of NCRNA00173 and its effect on OS remain unknown.

PI3K/Akt pathway has been considered to exert a crucial impact in tumor. [11–13] In OS, Ma H et al. [14] have revealed that LncRNA UCA1 promotes OS metastasis through EMT by activating PI3K/AKT/mTOR pathway. MDA19 has been reported to inhibit OS cell metastasis by PI3K/Akt/mTOR pathway. [15] However, whether NCRNA00173 influence OS via regulating PI3K/Akt/mTOR pathway is unclear.

In this study, we researched NCRNA00173 expression from 40 pairs of OS patients in children and analyzed the correlation between NCRNA00173 and clinical features. We further investigated the effect of NCRNA00173 in vitro and vivo. Our study discovered that NCRNA00173 was down-regulated and relevant with poor prognosis in pediatric OS, and overexpression of NCRNA00173 inhibited cell proliferation, migration and invasion via inhibition of PI3K/Akt pathway in OS. All of study may provide a new target for OS treatment.

Methods

Clinical samples

A total of 40 pairs of OS tissues aged from 4 to 16 years were collected in our hospital from December 2012 to November 2013. No patients were treated with chemotherapy or pre-operative radiotherapy. This study was ratified by the Ethics Committee of our hospital and it was complied with the Declaration of Helsinki. All of OS patients have signed informed consent.

Cell culture

The cell lines (U2OS, HOS, SaoS-2, NHOst) were obtained from the cell center of Shanghai (Shanghai, China). OS cells were cultured in RPMI1640 (Gibco, Grand Island, USA) culture medium containing 10% FBS(Gibco) and 1% streptomycin/penicillin (Gibco) at 37 °C with 5% CO_2.

Cell transfection

U2OS and HOS cells were grown in 6-well plate overnight, and separated into three groups: Mock group, pcDNA3.1 group and pcDNA3.1-NCRNA00173 group. The pcDNA3.1 group and pcDNA3.1-NCRNA00173 group of cells were transfected with negative control and pcDNA3.1-NCRNA00173 plasmid (Sangon Biotech Co., Ltd., Shanghai, China), respectively, using Lipofectamine® 2000 Reagent (Thermo Fisher scientific, Waltham, USA). Mock group was not treated. After 48 h of transfection, pcDNA3.1-NCRNA00173 group was cultured with RPMI1640 containing 20 nmol/L 740 Y-P (PI3K/Akt activator, MCE, China), which was named as pcDNA3.1-NCRNA00173 + 740 Y-P.

Cell proliferation assay

Cell proliferation was measured by MTT assay (Sigma, St. Louis, USA). The transfected U2OS and HOS cells were seeded in 96-well plates (5×10^3 per well). Then, cells were cultured for 24 h, 48 h and 72 h.

Then, MTT (5 mg/mL, 20 μL) was added. Finally, the absorbance was detected at 450 nm by microplate reader (Epoch2, Biotek, Vermont, USA).

Cell apoptosis assay by Flow Cytometry

After 48 h transfection of pcDNA3.1-NCRNA00173, U2OS and HOS cells were collected by centrifugation and washed with cool PBS. Then cell apoptosis was conducted by flow cytometry (FACS Calibur, BD Biosciences, Franklin Lakes, USA) using Annexin V-FITC apoptosis detection kit (BD Biosciences).

Wound-healing assay

Wound-healing assay was adopted to assess the ability of cell migration. After transfection, U2OS and HOS cells were cultured in 6-well plates (1×10^5 cells / well). The wound was obtained by using a 200 μl pipette tip on the center of the cell monolayer. Images were captured at 0 h and 48 h and assessed by ImageJ software.

Transwell assay

For migration and invasion assays, the transfected cells (1×10^5 cells / well) were seeded in the top chamber without / with matrigel (BD Bioscience). Then, RPMI1640 medium was added in the lower chamber. Transfected cells were grown for 48 h and migrated to the lower chamber. After 4% paraformaldehyde fixation, the migrated and invasive cells were stained with 0.1% crystal violet (Sigma). Finally, the amount of cells were calculated via microscope (Olympus Ckx53).

Quantitative real-Time PCR assays

Total RNA of tumor tissues of children from osteosarcoma and transfected cells was harvested by TRIZOL (Thermo Fisher scientific, USA). The cDNA was synthesized by reverse-transcribed kit (Trangene, Beijing, China). Then, qRT-PCR was conducted by SYBR RT-PCR kit (Thermo Scientific) and detected by LightCycler® 480 (Roche, Basel, Switzerland). β-actin was acted as internal control. The primer sequences used in this study were as follows: NCRNA00173 (forward): 5'-ACCTAGTCTTCCTTGGCACATC-3', (reverse): 5'-GGGATATTGATCTGAAGG

TGA-3'; β-actin (forward): 5'-ACACCTTCTACAATGAGCTG-3', (reverse): 5'-CTGCTTGCTGATCCACATCT-3'.

Western blot analysis

After transfection, protein was extracted by RIPA lysis buffer. Protein sample was added into 5 × loading buffer, boiled in water and then performed by SDS-PAGE. Following, the protein was transferred onto PVDF membrane and added into primary antibody (GAPDH, PI3K, Akt, p-Akt, 1:1000, CST, Boston, USA), (CyclinD1, P70S6K, P-P70S6K, VEGF, 1:1000, Abcam, Cambridge, USA) at 4 °C overnight. Following, HRP-conjugated secondary antibody (anti-rabbit IgG, 1:10000, Sigma) was incubated for 1 h. Finally, the bands of protein were conducted by ECL kit and quantized by Quantity One 1-D Analysis Software (Bio-Rad, Hercules, USA).

Animal experiments

Female nude mice (BALB/c, 4 weeks old, 18 ~ 22 g) were purchased from the center of Shanghai experimental animal. Animal experiments were lined with the institutional guidelines for the care and use of animals. The experiment was separated into two groups: pcDNA3.1 group and pcDNA3.1-NCRNA00173 group. Each group contained 10 nude mice. HOS cells (1×10^7 cells) of pcDNA3.1 and PCDNA3.1-NCRNA00173 group were injected into dorsal flanks of female nude mice. At the end of 4 weeks, the mice were sacrificed, and the tumor weight was measured. The tumor volume was calculated as $V = \text{Length} \times \text{width}^2 \times 0.5$.

Statistical analysis

Statistical analysis was performed by GraphPad Prism 6 software (La Jolla, USA) and SPSS 22.0 Statistical Software (Chicago, USA). Data were presented as mean ± standard deviation. The differences of groups were analyzed by Student's t-test and one-way ANOVA followed by Tukey's post hoctest were used to analyze two or multiple groups. All data were repeated three times. $P < 0.05$ was identified as remarkable difference.

Results

The expression of NCRNA00173 is down-regulated and relevant with poor prognosis in pediatric osteosarcoma

qRT-PCR was used to determine the relative NCRNA00173 expression in pediatric OS tissues and normal tissues. The expression of NCRNA00173 was notably reduced in OS tissues contrasted with adjacent normal tissues (Fig. 1A). Kaplan-Meier analysis revealed that overall survival (OS) of low NCRNA00173 expression was only 14%, and average survival time was 17 months within 5 years. Whereas, the OS of high NCRNA00173 expression was 26%, and average survival time was 32 months within 5 years (Fig. 1B). Besides, the correlation between NCRNA00173 expression and clinical characteristics in children were investigated. These data displayed that NCRNA00173 expression was conspicuously correlated with tumor metastasis and TNM stage (Table 1). Subsequently, Cox proportional hazard model verified that NCRNA00173 expression (for OS: RR 2.672, 95% CI, 1.03–6.93, P = 0.03), TNM stage (for OS: RR 4.971, 95% CI, 1.88–13.1, P = 0.001), metastasis (for OS: RR 0.333, 95% CI, 0.13–0.85, P = 0.01) were acted as independent prognostic factors in pediatric osteosarcoma (Table 2).

Table 1

Correlation between NCRNA00173 expression and clinical data in pediatric osteosarcoma

clinical data	No. of cases	NCRNA00173 expression		p-value
		High (n = 19)	Low (n = 21)	
Gender Male Female	22 18	10 9	12 9	0.66
Age, years < 13 ≥ 13	29 11	15 4	14 7	0.17
Tumor size, cm ≤ 5 > 5	20 20	12 7	8 13	0.12
TNM stage I–II III	29 11	18 1	11 10	0.0002**
Localization of the primary tumor Femur Tibia Humeral bone Other	8 10 12 10	3 5 6 5	5 5 6 5	0.96
Metastasis Present Absent	12 28	8 11	4 17	0.01*
*P < 0.05, **P < 0.01.				

Table 2

Multivariate survival analysis of overall survival in pediatric osteosarcoma

Variables	Overall survival		
	Exp (RR)	95% CI	P-value
Gender	1.045	0.429–2.548	0.922
Age Tumor size	1.904 1.832	0.633–5.726 0.76–4.41	0.25 0.1
NCRNA00173 TNM stage Metastasis	2.672 4.971 0.333	1.03–6.93 1.88–13.1 0.13–0.85	0.03* 0.001** 0.01*
*P < 0.05, **P < 0.01.			

Overexpression of NCRNA00713 inhibits OS cell proliferation and promotes cell apoptosis

Further, the expression of NCRNA00173 in OS cell lines was detected with qRT-PCR. The results revealed that NCRNA00173 expression in HOS, U2OS and Saos-2 was lower than that in NHOst ($P < 0.001$) (Fig. 2A), which was coincided with the consequence of OS tissues. After 48 h transfection, NCRNA00173 expression of pcDNA3.1-NCRNA00173 group was obviously increased compared with Mock and pcDNA3.1 group ($P < 0.01$) (Fig. 2B). The consequence of MTT indicated that overexpressed NCRNA00173 inhibited cell proliferation ability of U2OS and HOS cells at 48 and 72 h (Fig. 2C). While, comparing with Mock and pcDNA3.1 group, the apoptosis rate of U2OS and HOS cells was remarkable increased in pcDNA3.1-NCRNA00173 group ($P < 0.01$) (Fig. 2D). Therefore, these statistics testified that overexpression of NCRNA00173 restrained OS cell proliferation and accelerated cell apoptosis.

Overexpression of NCRNA00173 inhibits OS cell migration and invasion

The effects of NCRNA00173 on U2OS and HOS cells migration and invasion were performed by wound-healing and transwell assay. As shown in Fig. 3A, the ability of U2OS and HOS cells motility in pcDNA3.1-NCRNA00173 group was weaker than Mock and pcDNA3.1 group ($P < 0.01$). Meanwhile, the results of transwell assay revealed that the number of migration and invasion cells in the pcDNA3.1-NCRNA00173 group was dramatically declined contrasted with Mock and pcDNA3.1 group ($P < 0.01$) (Fig. 3B and Fig. 3C).

Overexpression of NCRNA00173 suppresses PI3K/Akt pathway in OS cells

PI3K/Akt pathway is considered to play a crucial impact on cell growth and cycle in cancer [16], and it has also been studied in osteosarcoma [17]. So, we explored whether NCRNA00173 influenced PI3K/Akt pathway in OS cells. Above of consequences indicated that the levels of p-PI3K and p-Akt in U2OS and HOS cells were markedly reduced in pcDNA3.1-NCRNA00173 group contrasted with Mock and pcDNA3.1 group ($P < 0.01$). However, no significantly changes were found of the total protein of PI3K and Akt ($P > 0.05$) (Fig. 4), suggesting that overexpression of NCRNA00173 suppressed PI3K/Akt pathway.

PI3K/Akt/mTOR pathway activator eliminates the role of NCRNA00173 in HOS

Whether the PI3K/Akt pathway is participated in the impact of NCRNA00173 in HOS cells. Thus, 20 μmol/l 740 Y-P (PI3K/Akt pathway activator was added into pcDNA3.1-NCRNA00173 group to specifically activate PI3K/Akt pathway, and we detected the interaction between 740 Y-P and NCRNA00173 on HOS cells. The consequences displayed that the upstream and downstream proteins of

PI3K/Akt/mTOR pathway (VEGF and Cyclin D1, P70S6K, p-p70S6k) in HOS cells were remarkably increased in pcDNA-NCRAN00173 + 740 Y-P group by comparison with pcDNA3.1-NCRNA00173 group ($P < 0.01$) (Fig. 5A). The cell proliferation, migrative and invasive capacity of HOS cells were higher in pcDNA-NCRAN00173 + 740 Y-P group than pcDNA3.1-NCRNA00173 group ($P < 0.05$) (Fig. 5B-D), but no obvious difference were discovered between pcDNA3.1 group and pcDNA-NCRAN00173 + 740 Y-P group ($P > 0.05$). Together, these consequences confirm that NCRNA00173 regulates PI3K/Akt pathway.

Overexpression of NCRNA00173 inhibits tumor in vivo

To farther discover the impact of NCRNA00173 in vivo, we injected HOS cells (pcDNA3.1 group and pcDNA3.1-NCRNA00173 group) into nude mice. The results showed that the tumor weight and volume in the pcDNA3.1-NCRNA00173 group were significantly smaller than those in the pcDNA3.1 group ($P < 0.01$) (Fig. 6A-C), indicating that NCRNA00173 inhibited the tumor growth in nude mice.

Discussion

OS is characterized by genetic instability including chromosomal rearrangement and gene mutations which originates from mesenchymal stem cells and has high mortality and disability rate in childhood and adolescent. [18,19] However, the molecular mechanism of osteosarcoma occurrence and progression has not been clarified yet. [20] In current study, the consequences of qRT-PCR explained that NCRNA00173 expression was down-regulated in pediatric OS tissues and cell lines. Notably, NCRNA00173 is significantly associated with clinical features, such as TNM stage and metastasis. We likewise discovered that overexpression of NCRNA00173 suppressed OS cell proliferation, migration and invasion via regulating PI3K/Akt signaling pathway, and inhibited tumor growth in vivo.

Until now, increasing evidences have indicated that LncRNAs play an important role in multiplex tumor biology, [21] for instance gastric cancer, colorectal cancer, and prostate cancer. [22–24] In previous study of OS, Cao K et al. [8] have demonstrated that high LncRNA HOXA11-AS has poor survival and is correlated with clinical stage, metastasis. Similarly, the expression of LncRNA XIST promotes OS progression and is associated with clinical features in OS patients, such as metastasis, clinical stage, and tumor size. Compared with lowly expressed of XIST group, the group of highly expressed has shorter overall survival in OS. [25,26] In this research, we found that NCRNA00173 was down-regulated in OS tissues and notably relevant with TNM stage and metastasis. Meanwhile, the OS and average survival time of low NCRNA00173 expression ware shorter than high NCRNA00173 expression within 5 years. Therefore, above consequences declared that the lower expression of NCRNA00173 was observably relevant with poor prognosis in OS.

PI3K/AKT pathway is one of the major downstream of VEGF and participates in cellular biological process. [27] Generally, PI3K could be induced by multiple factors, then activate the Akt and mTOR kinase in cancer cells. [28] mTOR has two distinct multiprotein complexes, which called mTORC1 and mTORC2. Moreover, mTORC1 directly phosphorylates S6K1, and p-S6K1 is involved in protein translation. [18]

Previous studies have revealed that the PI3K/Akt/mTOR pathway is particapted in the development of OS. For example, Li X et al. [29] have reported that LncRNA HOTAIR could promote cell progression through Akt/mTOR pathway in OS. Furthermore, according to reports demonstrated, MDA19 could suppress the downstream protein of PI3K/Akt/mTOR pathway in OS, such as Cyclin D1, P70S6K and p-P70S6K. [15] As a pathway activator, 740 Y-P could active PI3K. [30] Previous study has indicated that 740 Y-P could abolish silencing of LncRNA AB073614 on the role of cell proliferation and apoptosis in colorectal cancer. [31] Similarly, Zhang H et al. [32] has revealed that 740 Y-P also could elimate phellamurin on the effect of in OS. In our research, we discovered that overexpressed NCRNA00173 could inhibit the expression of p-PI3K and p-Akt, as well as 740 Y-P reversed the proteins level of VEGF, Cyclin D1, P70S6K and p-P70S6K and the proliferative, migrated and invasive capacity induced by NCRNA00173. These data suggested that NCRNA00173 could inhibit PI3K/Akt pathway in OS.

Conclusions

In conclusion, the current study elucidated that NCRBA00173 exhibited low expression in pediatric OS tissues and was related with poor prognosis. In addition, we further demonstrated that NCRNA00173 could significantly inhibit cell proliferation, migration and invasion through PI3K/Akt pathway in OS. Our research provides insights into molecular regulatory mechanism of OS and provides a reference to the treatment of OS in the future.

Declarations

LncRNA00173 (NCRNA00173)

Ethics approval and consent to participate: This study was conducted after obtaining approval of Liaocheng Dongchangfu District Maternal and Child Health Hospital’s ethical committee. Written informed consent was obtained from all subjects as well as parental consent for subjects aged less than 18 years.

Consent for publication: All authors approved to publish this study.

Availability of data and material: All data generated or analyzed during this study are included in this published article .

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable.

Authors' contributions: ZQZ and JCC designed and analyzed the experiment, and was a major contributor in writing the manuscript. DZX performed the experiment. All authors read and approved the final manuscript.

Acknowledgements: Not applicable.

References

1. Bielack SS, Hecker-Nolting S, Blattmann C, Kager L. Advances in the management of osteosarcoma. F1000Research. 2016;5:2767.
2. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. Cancer treatment and research. 2009;152:3-13.
3. PosthumaDeBoer J, Witlox MA, Kaspers GJ, van Royen BJ. Molecular alterations as target for therapy in metastatic osteosarcoma: a review of literature. Clinical & experimental metastasis. 2011;28:493-503.
4. Subbiah V, Kurzrock R. Phase 1 clinical trials for sarcomas: the cutting edge. Current opinion in oncology. 2011;23:352-60.
5. Bacci G, Rocca M, Salone M, Balladelli A, Ferrari S, Palmerini E, et al. High grade osteosarcoma of the extremities with lung metastases at presentation: treatment with neoadjuvant chemotherapy and simultaneous resection of primary and metastatic lesions. Journal of surgical oncology. 2008;98:415-20.
6. Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA biology. 2013;10:925-33.
7. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Molecular cell. 2011;43:904-14.
8. Cao K, Fang Y, Wang H, Jiang Z, Guo L, Hu Y. The lncRNA HOXA11-AS regulates Rab3D expression by sponging miR-125a-5p promoting metastasis of osteosarcoma. Cancer management and research. 2019;11:4505-18.
9. Lian H, Xie P, Yin N, Zhang J, Zhang X, Li J, et al. Linc00460 promotes osteosarcoma progression via miR-1224-5p/FADS1 axis. Life sciences. 2019;233:116757.
10. Fang TT, Sun XJ, Chen J, Zhao Y, Sun RX, Ren N, et al. Long non-coding RNAs are differentially expressed in hepatocellular carcinoma cell lines with differing metastatic potential. Asian Pacific journal of cancer prevention : APJCP. 2014;15:10513-24.
11. Sharma V, Sharma AK, Punj V, Priya P. Recent nanotechnological interventions targeting PI3K/Akt/mTOR pathway: A focus on breast cancer. Seminars in cancer biology. 2019.
12. Guerrero-Zotano A, Mayer IA, Arteaga CL. PI3K/AKT/mTOR: role in breast cancer progression, drug resistance, and treatment. Cancer metastasis reviews. 2016;35:515-24.
13. Li H, Zeng J, Shen K. PI3K/AKT/mTOR signaling pathway as a therapeutic target for ovarian cancer. Archives of gynecology and obstetrics. 2014;290:1067-78.
14. Ma H, Su R, Feng H, Guo Y, Su G. Long noncoding RNA UCA1 promotes osteosarcoma metastasis through CREB1-mediated epithelial-mesenchymal transition and activating PI3K/AKT/mTOR pathway. Journal of bone oncology. 2019;16:100228.

15. Liu B, Xu L, Dai EN, Tian JX, Li JM. Anti-tumoral potential of MDA19 in human osteosarcoma via suppressing PI3K/Akt/mTOR signaling pathway. Bioscience reports. 2018;38.
16. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR Signaling in Cancer. Frontiers in oncology. 2014;4:64.
17. Niu NK, Wang ZL, Pan ST, Ding HQ, Au GH, He ZX, et al. Pro-apoptotic and pro-autophagic effects of the Aurora kinase A inhibitor alisertib (MLN8237) on human osteosarcoma U-2 OS and MG-63 cells through the activation of mitochondria-mediated pathway and inhibition of p38 MAPK/PI3K/Akt/mTOR signaling pathway. Drug design, development and therapy. 2015;9:1555-84.
18. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer cell. 2007;12:9-22.
19. Munoz P, Iliou MS, Esteller M. Epigenetic alterations involved in cancer stem cell reprogramming. Molecular oncology. 2012;6:620-36.
20. Broadhead ML, Clark JC, Myers DE, Dass CR, Choong PF. The molecular pathogenesis of osteosarcoma: a review. Sarcoma. 2011;2011:959248.
21. Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. Cancer cell. 2016;29:452-63.
22. Isin M, Uysaler E, Ozgur E, Koseoglu H, Sanli O, Yucel OB, et al. Exosomal lncRNA-p21 levels may help to distinguish prostate cancer from benign disease. Frontiers in genetics. 2015;6:168.
23. Li H, Yu B, Li J, Su L, Yan M, Zhu Z, et al. Overexpression of lncRNA H19 enhances carcinogenesis and metastasis of gastric cancer. Oncotarget. 2014;5:2318-29.
24. Zheng HT, Shi DB, Wang YW, Li XX, Xu Y, Tripathi P, et al. High expression of lncRNA MALAT1 suggests a biomarker of poor prognosis in colorectal cancer. International journal of clinical and experimental pathology. 2014;7:3174-81.
25. Li GL, Wu YX, Li YM, Li J. High expression of long non-coding RNA XIST in osteosarcoma is associated with cell proliferation and poor prognosis. European review for medical and pharmacological sciences. 2017;21:2829-34.
26. Yang C, Wu K, Wang S, Wei G. Long non-coding RNA XIST promotes osteosarcoma progression by targeting YAP via miR-195-5p. Journal of cellular biochemistry. 2018;119:5646-56.
27. Rini BI, Small EJ. Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23:1028-43.
28. Wang SF, Chou YC, Mazumder N, Kao FJ, Nagy LD, Guengerich FP, et al. 7-Ketocholesterol induces P-glycoprotein through PI3K/mTOR signaling in hepatoma cells. Biochemical pharmacology. 2013;86:548-60.
29. Li E, Zhao Z, Ma B, Zhang J. Long noncoding RNA HOTAIR promotes the proliferation and metastasis of osteosarcoma cells through the AKT/mTOR signaling pathway. Experimental and therapeutic medicine. 2017;14:5321-8.
30. Derossi D, Williams EJ, Green PJ, Dunican DJ, Doherty P. Stimulation of mitogenesis by a cell-permeable PI 3-kinase binding peptide. Biochemical and biophysical research communications.

1998;251:148-52.

31. Wang Y, Kuang H, Xue J, Liao L, Yin F, Zhou X. LncRNA AB073614 regulates proliferation and metastasis of colorectal cancer cells via the PI3K/AKT signaling pathway. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2017;93:1230-7.
32. Zhang H, Jiang H, Zhang H, Liu J, Hu X, Chen L. Anti-tumor efficacy of phellamurin in osteosarcoma cells: Involvement of the PI3K/AKT/mTOR pathway. European journal of pharmacology. 2019;858:172477.

Abbreviations

Osteosarcoma (OS)

Figures

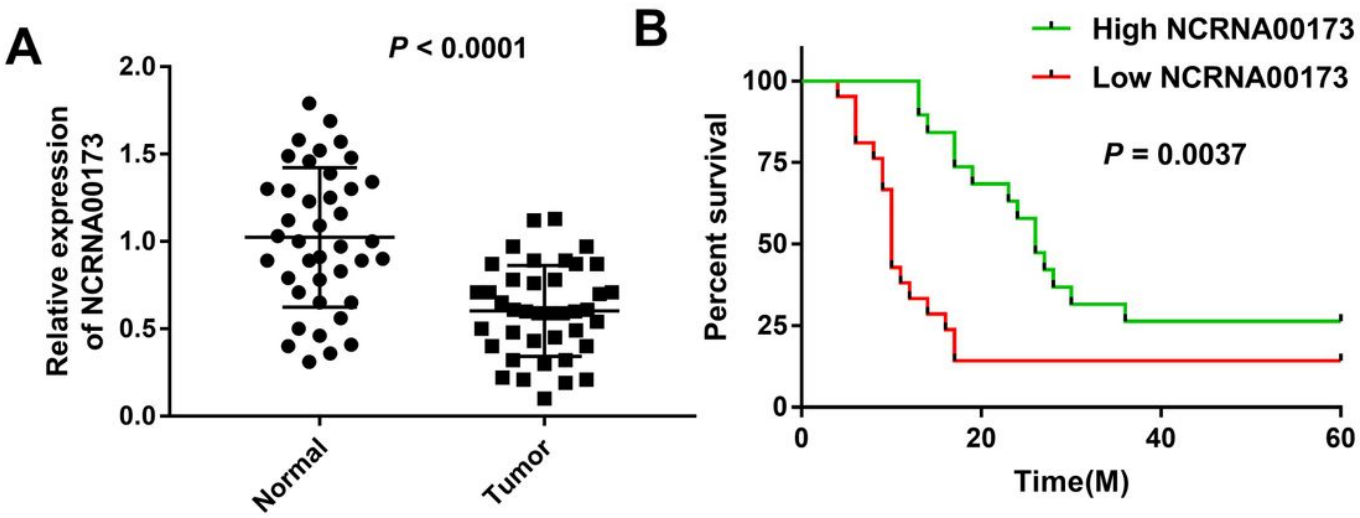

Figure 1

NCRNA00173 was down-regulated and correlated with poor prognosis in pediatric osteosarcoma. (A) The expression of NCRNA00173 was detected by qRT-PCR in pediatric osteosarcoma tissues and adjacent normal tissues. (B) Kaplan-Meier analyzed between NCRNA00173 expression and overall survival of pediatric osteosarcoma. Values are presented as mean ± standard deviation with repeated for three times. P < 0.0001 compared with Normal group.

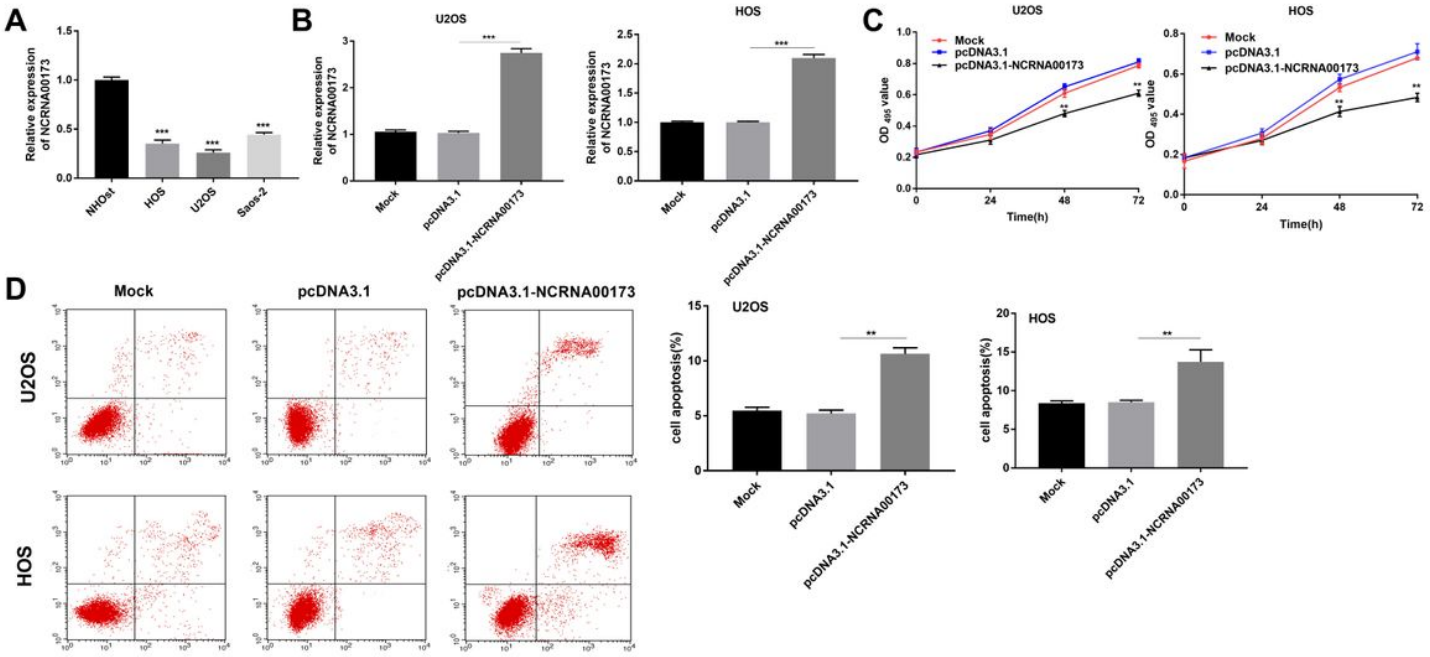

Figure 2

Overexpression of NCRNA00713 inhibited OS cell proliferation and promoted cell apoptosis. (A) NCRNA00713 expression was detected by qRT-PCR in OS cell lines (HOS, U2OS and Saos-2) and human osteoblast cell lines (NHOst). (B) The expression of NCRNA00713 was detected by qRT-PCR in U2OS and HOS cells transfected with pcDNA3.1 and pcDNA3.1-NCRNA00173. (C) NCRNA00173 effect on U2OS and HOS cells proliferation after incubation at 0, 24, 48, 72 h. (D) The cell apoptosis of U2OS and HOS cells was detected by flow cytometry after transfection with NCRNA00173.Values are presented as mean ± standard deviation with repeated for three times. **P < 0.01, ***P < 0.001 compared with Mock and pcDNA3.1 group.

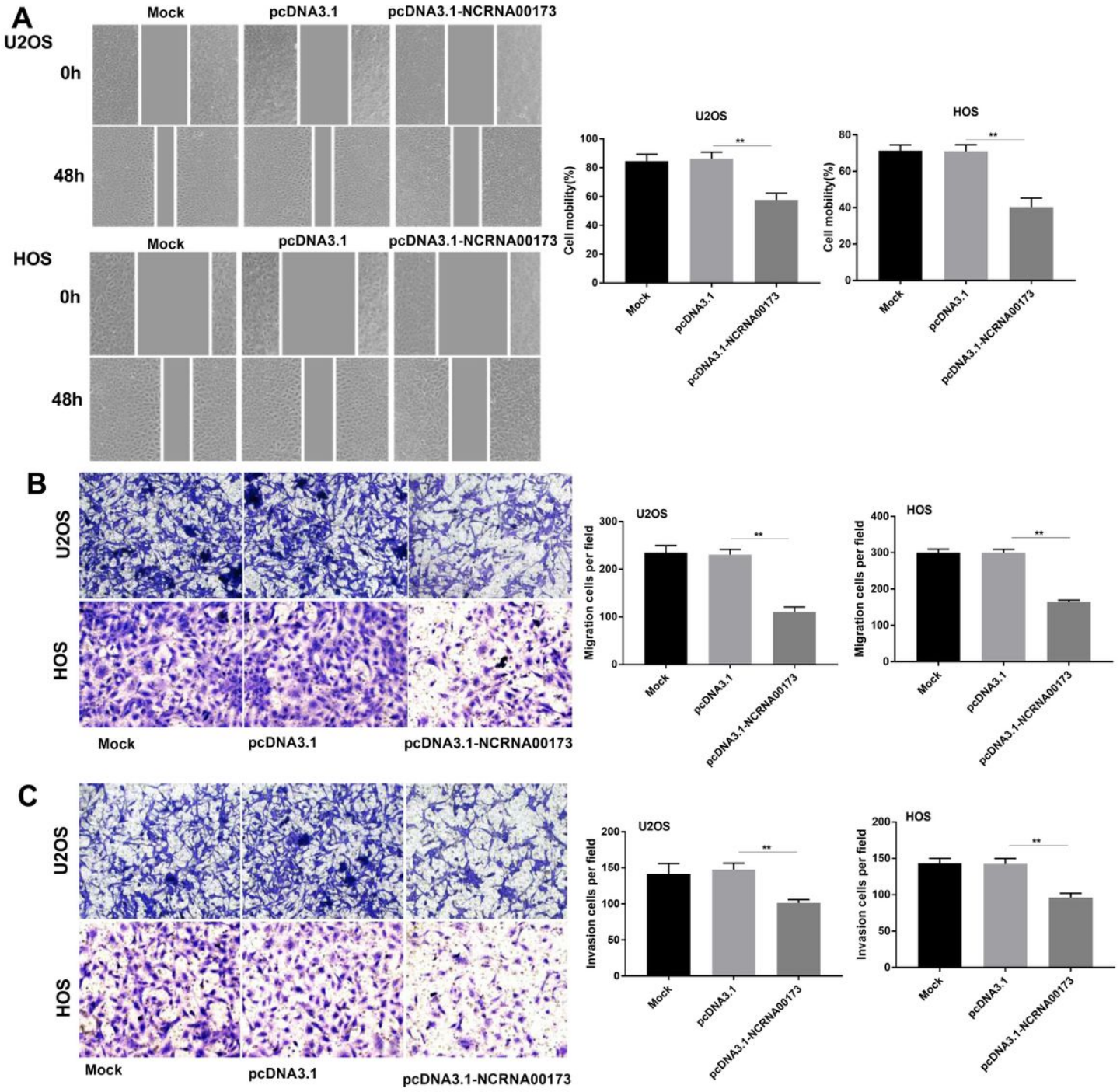

Figure 3

Overexpression of NCRNA00173 inhibited OS cell migration and invasion. (A) The ability of cell motility was test by wound-healing assay after treatment NCRNA00173 in U2OS and HOS cells. (B) The number of migrated cells was tested by transwell assay after treatment NCRNA00173 in U2OS and HOS cells. (C) The number of invasive cells was tested by transwell assay after treatment NCRNA00173 in U2OS and HOS cells. Values are presented as mean ± standard deviation with repeated for three times. **P < 0.01 compared with Mock and pcDNA3.1 group.

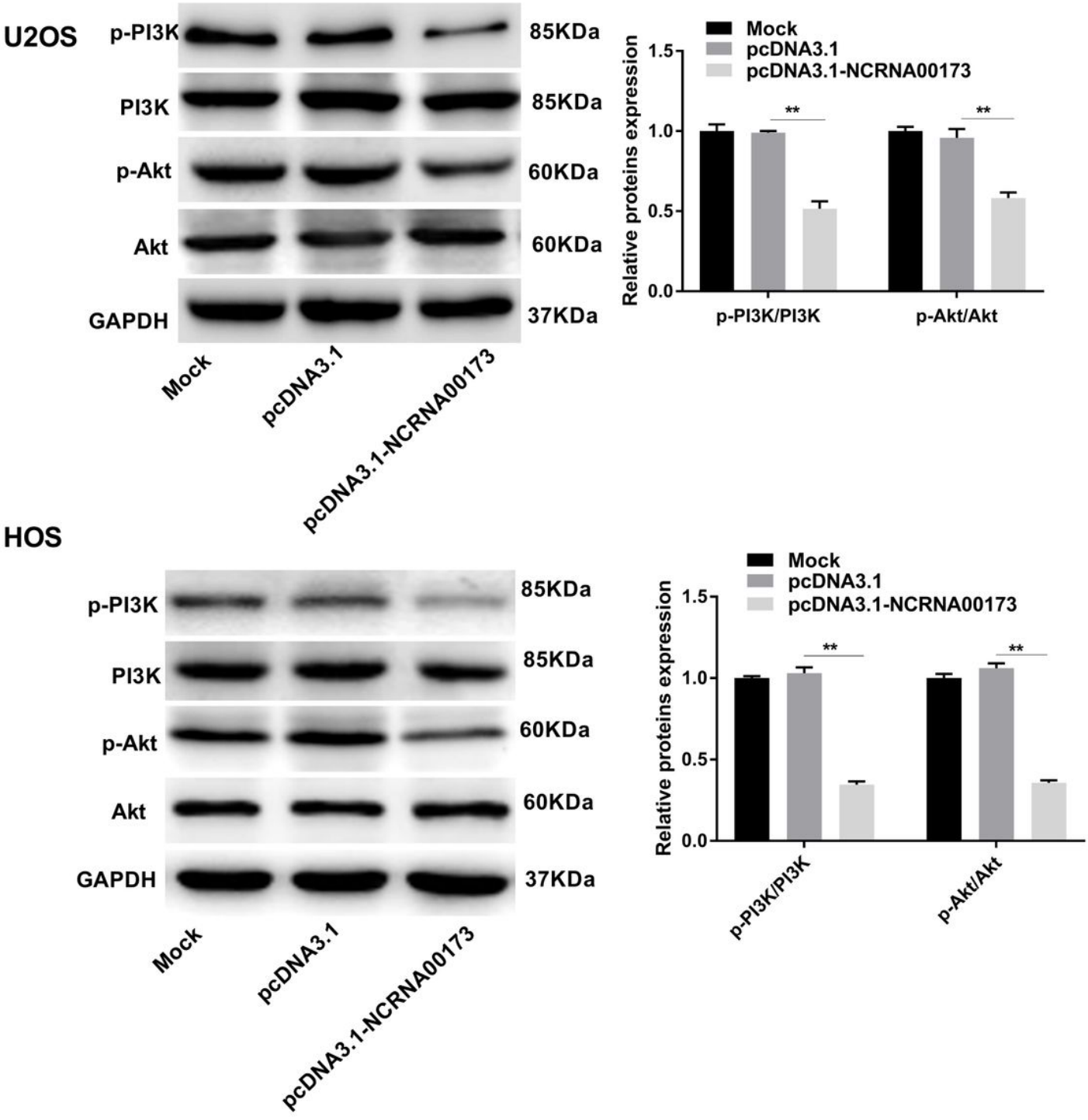

Figure 4

Overexpression of NCRNA00173 suppressed PI3K/Akt pathway in OS cells. Note: The protein of p-PI3K and p-Akt total protein of PI3K, Akt was detected by western blot after treatment NCRNA00173 in U2OS and HOS cells. Values are presented as mean ± standard deviation with repeated for three times. *P < 0.01compared with Mock and pcDNA3.1 group.

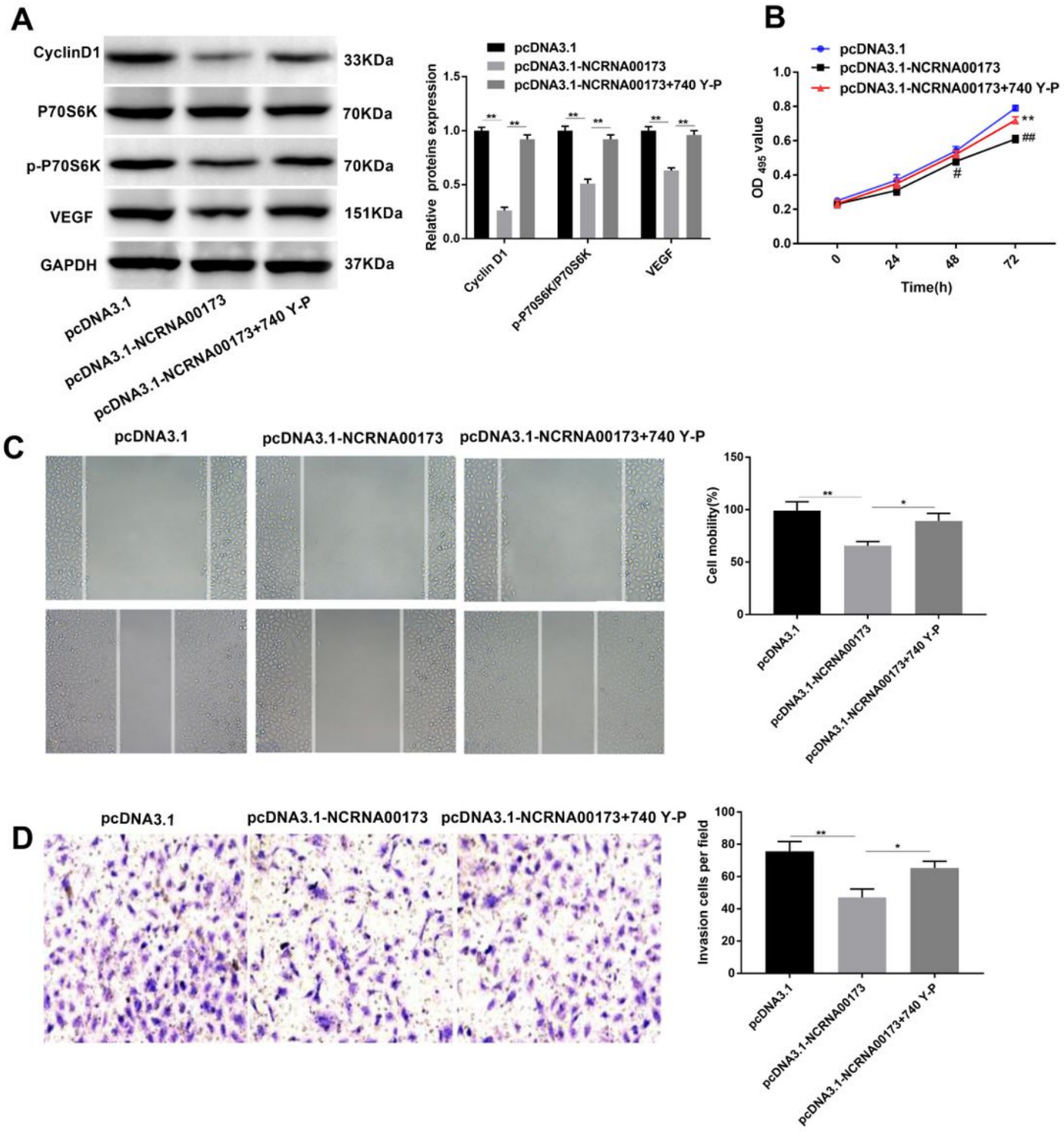

Figure 5

740 Y-P (PI3K/Akt pathway activator) eliminated the role of NCRNA00173 in HOS cells. (A) Interaction between 740 Y-P and NCRNA00173 on the downstream and upstream proteins of PI3K/Akt pathway (Cyclin D1, P70S6K, p-p70S6K and VEGF) in HOS cells was detected by western blot. (B) Interaction between 740 Y-P and NCRNA00173 on the ability of cell proliferation in HOS cells was detected by MTT assay. (C) Interaction between 740 Y-P and NCRNA00173 on the ability of migration and invasion (D) in

HOS cells was detected by transwell assay. Values are presented as mean ± standard deviation with repeated for three times. *P < 0.05, **P < 0.01, compared with pcDNA3.1 and pcDNA3.1-NCRNA00173 group.

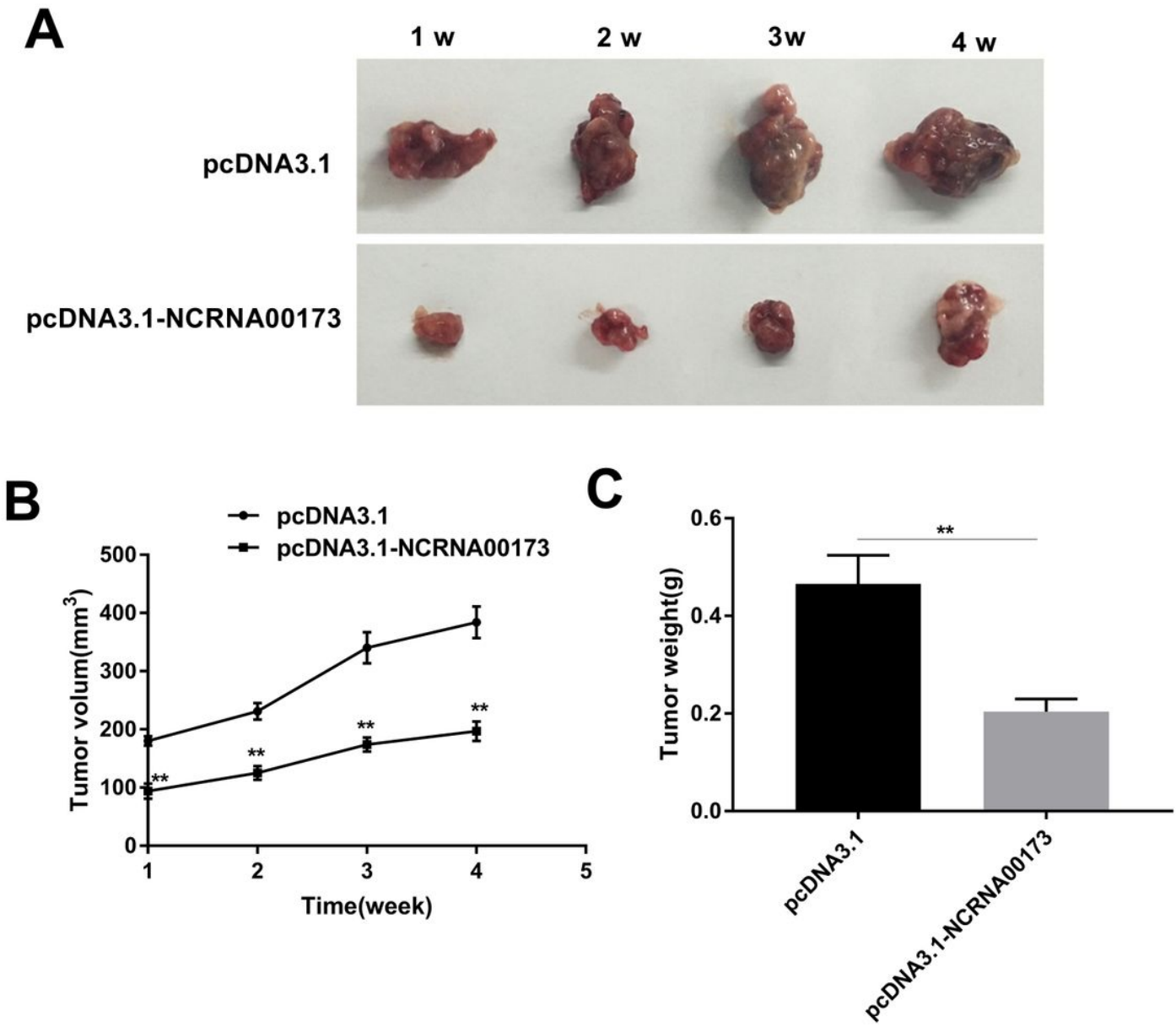

Figure 6

Overexpression of NCRNA00173 inhibited tumor in vivo. (A) Tumor was measured at 4 weeks after injecting HOS cells transfected by pcDNA3.1 and pcDNA3.1-NCRNA00173 into nude mice. (B) Tumor volume and weight (C) were measured after injecting HOS cells transfected by pcDNA3.1 and pcDNA3.1-NCRNA00173 into the nude mice. Values are presented as mean ± standard deviation with repeated for three times. **P < 0.01 compared with pcDNA3.1 group.