Pathology - Research and Practice 215 (2019) 152381

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Tankyrase1 antisense oligodeoxynucleotides suppress the proliferation, migration and invasion through Hippo/YAP pathway in human osteosarcoma cells

Yichi Zhou, Qi Jin, Wei Xiao, Chengjun Sun*

Department of Orthopaedics, CR & WISCO General Hospital, Wuhan, 430000, PR China

ARTICLE INFO

Keywords:
Osteosarcoma
Tankyrase1
Antisense oligodeoxynucleotides
Epithelial-mesenchymal transition
Hippo
YAP

ABSTRACT

Osteosarcoma is the most common malignant tumor of bone with a high potential for metastasis and poor prognosis. This study intends to explore the effect of tankyrase1 (TANK1) in the development of osteosarcoma cells and the underlying mechanism. The osteosarcoma cell line MG-63 cells were cultured and transfected with tankyrase1 antisense oligodeoxynucleotides (TANK1-ASODN). Cell proliferation was detected with CCK-8 and immunofluorescence. Cell migration and invasion were examined by wound healing assay and Transwell assay, respectively. Reverse transcription-quantitative polymerase chain reaction was performed to detect the mRNA level of TANK1 and western blot was conducted to detect relative protein expression during the research. As a result, we demonstrated that TANK1 was upregulated in osteosarcoma. The TANK1-ASODN inhibited MG-63 cell proliferation, migration and invasion. The progress of epithelial-mesenchymal transition (EMT) was also suppressed in TANK1-ASODN transfected MG-63 cells compared to control group. Besides, the TANK1-ASODN activated and modulated the Hippo/YAP signaling which might be the pathway that TANK1 depended on. Overall, our finding supported that TANK1-ASODN slowed down the progress of osteosarcoma by suppressing cell proliferation, migration, invasion and EMT through Hippo/YAP pathway.

1. Introduction

Osteosarcoma is the most common histological form of primary bone cancer which is widespread in children and adolescents [1], and the precise pathogeny still remains unclear. For lack of specific symptoms, early diagnose is commonly delayed. Cancer metastasis, which is a major cause of cancer deaths, has been found in osteosarcoma patients up to 23% when they are diagnosed [2,3]. Although the development in diagnosis and treatment related with surgical techniques and adjuvant chemotherapy of osteosarcoma in recent years, 5-year survival rates of osteosarcoma patients remains unsatisfactory [4]. Thus, it is imperatively needed to explore osteosarcoma progression mechanisms and seek for its potential diagnosed targets and therapeutic targets.

Chromosomal instability may play a vital role in the early phases of carcinogenesis in spite of great heterogeneity of the molecular and biological features [5]. Telomerase and telomeres take great responsible for chromosomal stability, cell multiplication and integrity. Telomeres are highly conserved structures located at the ends of eukaryotic chromosomes that comprised of tandem repeats of a DNA sequence [6]. The functions of telomeres mainly include preventing double strand break DNA damage signals and protecting chromosomes from end-to-end fusion and degradation [7]. Telomeres are getting shorter during cell division, and when telomeres become critically short, they are unable to protect DNA replication, and then cells undergo senescence and apoptosis [8,9]. Thus the length of telomeres is closely associated with cell deaths. Telomerase, a nucleoprotein enzyme, has transcriptase activity and maintains telomere length after each replication cycle [10]. Telomerase-mediated telomeric repeat binding factor1 (TRF-1), a negative regulatory factor, cooperates with telomerase functioning as a positive regulatory factor to maintain dynamic equilibrium of telomere length [11].

Tankyrases (tankyrase1/TANK1 and tankyrase2/TANK2) are critical to cancer cell growth as it functions on telomere cohesion and length homeostasis [12]. Meanwhile, TANK1 is identified as a telomere-associated protein that binds to the telomere-specific DNA binding protein TRF1 and co-localizes with TRF1 at the ends of chromosomes in metaphase, regulating telomere elongation [13]. Thus, TANK1 is considered as a positive regulator of telomere length [11], and its RNA interference leads to telomere shortening proportional to the level of disintegration [14]. TANK1 plays a vital role in chromosomal stability

* Corresponding author at: CR & WISCO General Hospital, Yejin Avenue 29, Qingshan District, Wuhan, 430000, PR China.
E-mail address: scj18185@sina.com (C. Sun).

https://doi.org/10.1016/j.prp.2019.03.010
Received 20 December 2018; Received in revised form 12 February 2019; Accepted 4 March 2019

and cell growth. To date, abnormally high expressed TANK1 has been discovered in more and more human cancers, such as breast cancer, colon cancer and so on [15,16]. However, the research concerned on TANK1 and osteosarcoma is little, although TANK1 has become a hallmarks of malignant tumors.

As above, our study aimed to investigate the effect of TANK1, and explore its potential mechanism in osteosarcoma.

2. Methods

2.1. Cell culture

Three osteosarcoma cell lines (U-2OS and MG-63) and a human osteoblast cell line hFOB 1.19 were purchased from American Type Culture Collection (Manassas, VA, USA). hFOB 1.19 cells were cultured in a 1:1 mixture of Ham's F12 Medium (GE Healthcare Life Sciences, USA) and Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA) with 10% foetal bovine serum (FBS; Gibco, USA) and 1% PS (penicillin and streptomycin, Gibco, USA). The remaining cells were cultured in DMEM supplemented with 10% FBS and 1% PS. All cell lines were cultured at 37°C in a 5% CO_2 cell culture incubator.

2.2. Cell transfection

MG-63 cells were cultured in 6-well plates for 24 h. Then cells were transfected by TANK1-ASODN or negative control (NC) using Lipofectamine 2000 (Invitrogen, USA) according to manufacturer's instructions. After 48 h, all the transfected cells were further analyzed.

2.3. Cell proliferation assay

MG-63 cells transfected with TANK1-ASODN were harvested and seeded in 96-well plates at a density of 4×10^3 cells/well. Then, cell proliferation ability of cells was examined by the Cell Counting Kit-8 kit (CKK-8; Beyotime Institute of Biotechenology, China) in accordance with the manufacturer's protocol. The absorbance of each well was determined at 450 nm using a microplate reader (Bio-Tek Instruments, Inc., USA) when the reagent reacted for 24, 48 and 72 h at room temperature. All experiments were performed in triplicate.

2.4. 4',6-Diamidibno-2-phenylindole (DAPI) staining

Cells were grown in 6-well plates, washed with ice-cold PBS, fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.5% Triton X-100 at room temperature. For nuclear morphology determination, DAPI was used to stain cell nuclei for 5 min in the dark. A fluorescence microscope (Olympus, Japan) was used to observe and record positive staining. All experiments were performed in triplicate.

2.5. Cell migration assay

Cell migration ability of cells was determined using a wound healing assay. Briefly, cells were cultured at 5×10^4 cells/well in 24-well plates and were incubated overnight at 37 °C to allow the cells adhere. After the cell achieved a confluence, a wound was generated with a pipette tip and the cultured media was changed to remove detached cells. Then we photographed the scratched area with a microscope. All experiments were performed in triplicate.

2.6. Cell invasion assay

Cell invasive ability of cells was assessed by transwell assay. Briefly, cells were cultured in DMEM without serum in the upper chamber coated with 50 μL of 1 g/L Matrigel (BD Biosciences, USA) and the complete medium was added into the lower chamber. 24 h later, the invasive cells were fixed with methanol and stained with 0.1% crystal violet. Then the stained cells were counted from five randomly selected areas per well. All experiments were performed in triplicate.

2.7. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from cells using TRIzol reagent (Thermo Fisher Scientific, Inc. USA) according to the manufacturer's instruction. Subsequently the first-strand complementary DNA was produced by reverse transcriptase (Takara, Japan) in accordance with the manufacturer's protocol. Subsequently, qPCR was performed by using Power SYBR Green master Mix (Life Technology Inc., USA) and the LightCycler PCR (Roche Diagnostics, USA) to quantify the mRNA levels. The primers used in the PCR reaction are TANK1 forward primer (5′-ATGCCCCCAGAGGCCTTAC-3′) and reverse primer (5′-GGTGGATG CTGGTGA GATCA-3′); and β-actin forward primer (5′-CCACACTGTGC CCATCTACG-3′) and reverse primer (5′-AGGATCTTCATGAGGTAGTC AGTCAG-3′). TANK1 expression was normalized using the $2^{-\triangle\triangle Ct}$ method relative to the mRNA expression of β-actin. All experiments were performed in triplicate.

2.8. Western blot analysis

Cells were collected, washed and lysed with the Total Protein Extraction Kit (Applygen Technologies Inc., China). Then the protein concentration was detected with a BCA Protein Assay Kit (Thermo Fisher Scientific, Inc., USA). Equal amounts of protein were resolved on a 10% SDS-PAGE gel and transferred to polyvinylidene fluoride (PVDF) membrances (Millipore, USA). The blots were blocked with 5%BCA (Sigma-Aldrich, German) at room temperature for 1 h and incubated with the primary antibodies. After washing three times with tris-buffered saline and Tween 20, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody and visualized using SuperSignal West Dura Extended Duration Substrate kit (Thermo Fisher Scientific, Inc., USA). All experiments were performed in triplicate.

2.9. Statistical analysis

Data were analyzed using GraphPad Prism version 6.0 (GraphPad software, Inc., USA) and SPSS statistical software version 20.0 (SPSS, Inc., USA). All data are expressed as the mean ± SD. A Student's *t*-test and one-way ANOVA were used for statistical analyses. $P < 0.05$ was considered to a statistically significant difference.

3. Results

3.1. TANK1 was up-regulated in osteosarcoma

In order to investigate the role of TANK1 in osteosarcoma, the relative transcript level of TANK1 in hFOB 1.19, U-2OS and MG-63 cells was examined (Fig. 1). Compared with the normal cells, osteosarcoma cells exhibited higher expression of TANK1. Of note, MG-63 cells exhibited highest expression of TANK1, so we selected MG-63 cells for the next research.

3.2. TANK1-ASODN in osteosarcoma inhibited cell proliferation in vitro

To investigate the role of TANK1 in MG-63 cell proliferation, MG-63 cells were transfected with TANK1-ASODN or the NC control. The growth curve of MG-63 cells was drawn by CCK-8 method. As it revealed in Fig. 2A, the data revealed that there was no significant difference in the proliferation rate between MG-63 cells control group and negative control group. While TANK1-ASODN group exhibited notably decrease in cell proliferation compared with control groups. Immunofluorescence assay also exhibited a great decrease of cell proliferation in

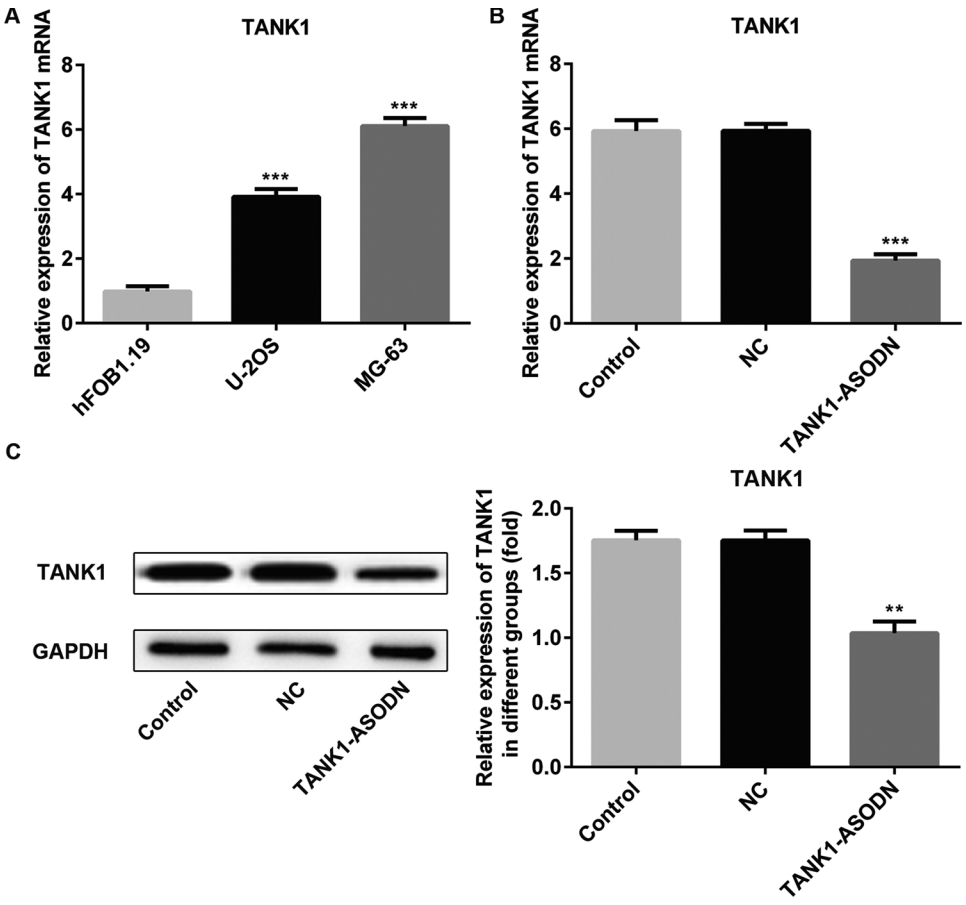

Fig. 1. TANK1 was up-regulated in osteosarcoma.
(A) The relative transcript level of TANK1 in hFOB 1.19, U-2OS, MG-63 and SW1353 cells was examined by RT-qPCR. Data is presented as the mean ± SD of three experiments. ***P < 0.001 vs hFOB 1.19 cells. After transfection by TANK1-ASODN or NC in MG-63 cells, the level of TANK1 was detected by RT-qPCR (B) and WB (C). Data is presented as the mean ± SD of three experiments. ***P < 0.001 vs control.

TANK1-ASODN group, compared with other groups (Fig. 2B).

Additionally, expression of cyclin-dependent kinase 2 (CDK-2), cyclin-dependent kinase inhibitor 1(p21) and cell cyclin relative protein Cyclin E1 was detected by western blot to verify the effect of TANK1-ASODN on cell proliferation further. As was shown in Fig. 2C–D, protein expressions of CDK-2 and Cyclin E1 were down-regulated and p21 was up-regulated in TANK1-ASODN transfected cells compared to control groups. These results suggested that TANK1-ASODN could suppress cell proliferation in osteosarcoma.

3.3. TANK1-ASODN suppressed cell migration and invasion in osteosarcoma

We next investigated the effect of TANK1-ASODN on MG-63 cell

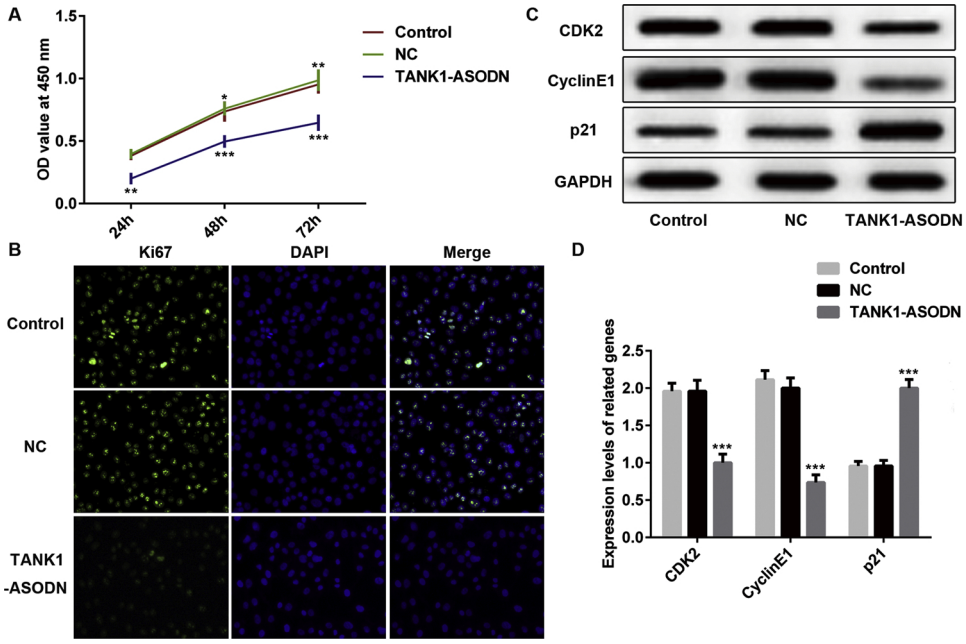

Fig. 2. TANK1-ASODN inhibited cell proliferation in osteosarcoma.
(A) After transfection, the proliferation curve of MG-63 cells was determined by CKK-8 assay. OD values at 450 nm were detected at 24 h, 48 h and 72 h, respectively. (B) DAPI staining were performed to examine cell proliferation. (C) The protein expressions of CDK2, cyclin E1 and p21 were determined by western blot, and were quantified (D). Data is presented as the mean ± SD of three experiments. **, ***P < 0.01, 0.001 vs control.

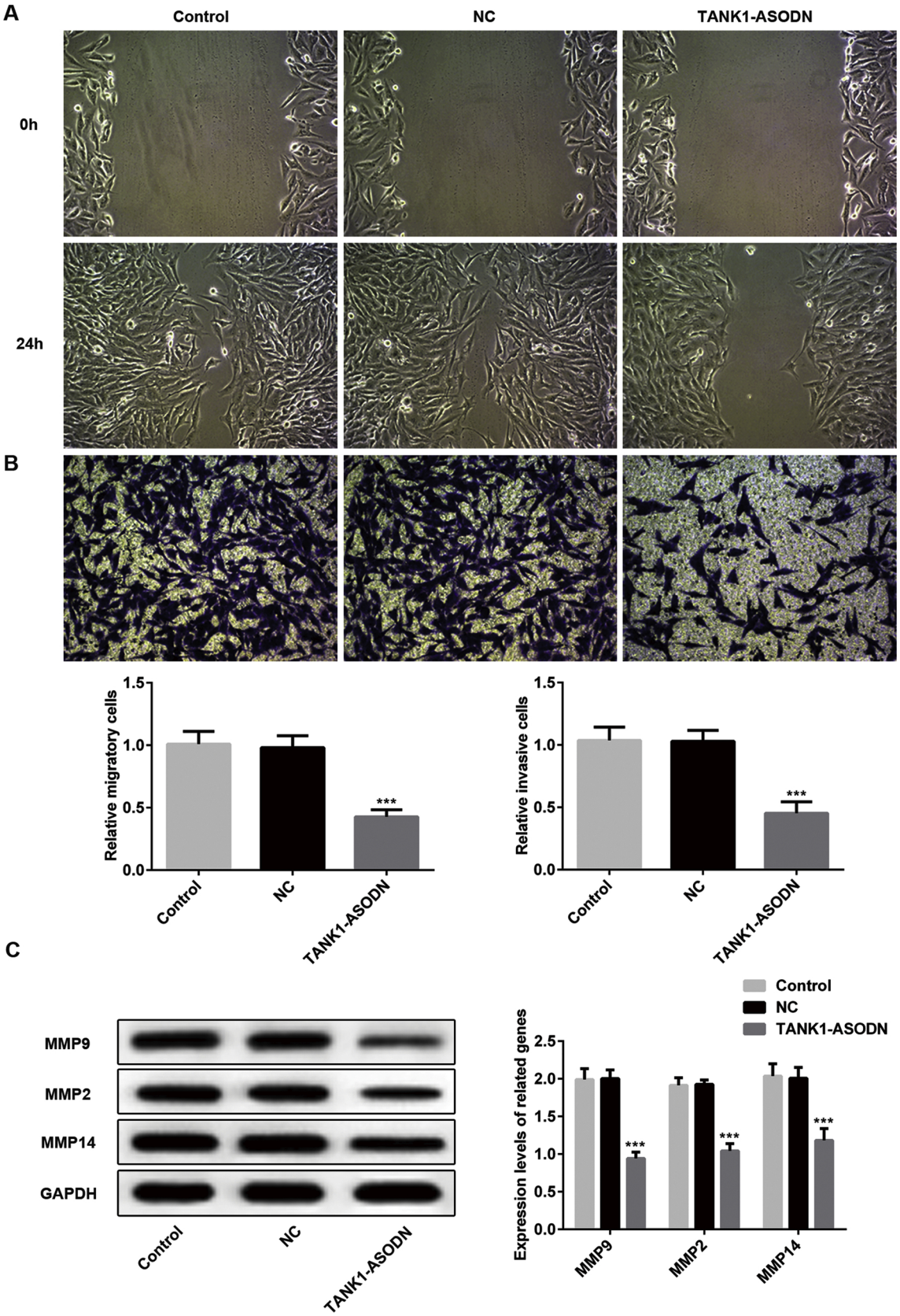

Fig. 3. TANK1-ASODN suppressed cell migration and invasion in osteosarcoma.
(A) Wound healing assay was performed to detect cell migration ability. (B) Transwell assay was performed to detect cell invasive ability. (C) Cell migration ability was quantified. (D) Cell invasive ability was quantified. (E) The protein expressions of MMP2, MMP9 and MMP14 were detected by western blot and quantified. Data is presented as the mean ± SD of three experiments. ***P < 0.001 vs control.

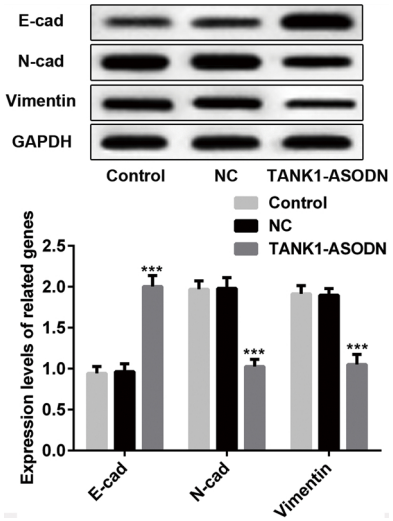

Fig. 4. TANK1-ASODN suppressed EMT in osteosarcoma.
The protein expressions of EMT relative protein, E-cadherin, N-cadherin and vimentin were examined by western blot, and the protein bands were quantified. Data is presented as the mean ± SD of three experiments. ***P < 0.001 vs control.

migration and invasion. A wounding heal assay demonstrated that TANK1-ASODN clearly inhibited the migration capacity of MG-63 cells (Fig. 3A, C). Similarly, a Transwell assay suggested that the invasive capacity was significantly suppressed in cells transfected with TANK1-ASODN compared with the control groups (Fig. 3B, D). Furthermore, expression of migration relative protein MMP9, MMP2 and MMP14 was obviously downregulated in TANK1-ASODN transfected cells. These findings indicated that TANK1-ASODN could inhibit the migration and invasion of MG-63 cells.

3.4. TANK1-ASODN reversed Epithelial-Mesenchymal Transition (EMT) in osteosarcoma

To Figure out the role of TANK1-ASODN in EMT of MG-63 cells, the expression of EMT relative proteins E-cadherin, N-cadherin and vimentin was detected. Western blot showed that TANK1-ASODN induced the E-cadherin protein expression and suppressed the protein expression of N-cadherin and vimentin (Fig. 4).

3.5. TANK1-ASODN activated the Hippo/YAP signaling

To explore the potential mechanism of TANK1-ASODN in suppressing the proliferation, migration, invasion and EMT of MG-63 cells, we screened Hippo/YAP signaling as potential target gene. The data shown in Fig. 5 indicated that the expression of phosphorylated YAP1 exhibited an increase in MG-63 cells that were transfected with TANK1-ASODN, as compared with control cells. Meanwhile, the expression levels of the Hippo upstream gene, MST1 and LATS1 were examined. The results showed that there was no significant difference in expression levels of MST1 between TANK1-ASODN transfected cells and control cells, whereas the expression of LATS1 was prominently increased in TANK1-ASODN transfected cells. These data revealed that TANK1-ASODN could activate the Hippo/YAP pathway to suppress cell proliferation, migration, invasion and EMT in osteosarcoma.

4. Discussion

Tankrases, also known as PARP5a/ARTD5 and PARP5b/ARTD6, are members of the poly (ADP-ribose) polymerase (PARP) family proteins, which have PARP catalytic domains. Overexpressed TANK1 decreased the genotoxic-induced cell death by inhibiting PARP1 [17]. Besides, TANK1 exert function in cellular pathways that are critical to cancer cell growth including telomere cohesion and length homeostasis, Wnt/β-catenin signaling, and mitotic progression [12,18]. TANK1 was considered as tumor antigens and potential targets of anticancer therapy in recent studies [19,20]. Thus, our study was designed to investigate the actual effect of TANK1 in human osteosarcoma.

Firstly, we analyzed the expression levels of TANK1 in osteosarcoma cells and normal cells. We found that TANK1 level was dramatically unregulated in the osteosarcoma cells. Besides, TANK1 played an important role in osteosarcoma cell proliferation, metastasis and invasion. Progression through the cell cycle was dependent on the activation of CDKs and their regulatory subunits, the cyclins [21]. In this study, we observed that TANK1-ASODN significantly suppressed MG-63 cell growth by downregulating CDK2, Cyclin E1 and upregulating p21. In addition, TANK1-ASODN notably decreased cell migration and invasion in MG-63 cells. These properties might contribute to the TANK1-linked aggressive biological behaviours of osteosarcoma.

EMT, involved in the conversion of a cancer cell from its epithelial form to its motile mesenchymal form, was considered as an initial and crucial role in the metastasis and invasion of tumor cells [22,23]. Thus we hypothesized that TANK1 might influence EMT in osteosarcoma caner. Our results demonstrated that TANK1 expression level was negatively correlated with epithelial marker (E-cadherin), but was positive associated with mesenchymal marker (N-cadherin and vimentin), which suggested that TANK1 could influence EMT to promote the metastasis and invasion of MG-63 cells and aggravate osteosarcoma. However, it could be reversed by TANK1-ASODN as in TANK1-ASODN cells, the level of E-cadherin was elevated and the level of N-cadherin and vimentin was decreased, indicating that TANK1-ASODN could inhibit the progression of EMT, consequently suppressing the tumor aggressiveness, consistent with its inhibitor effect on cell proliferation, migration and invasion in MG-63 cells.

The Hippo pathway has exhibited tumor suppressive in various cancers. The core kinase cassette of the mammalian Hippo pathway includes STE20 family protein (MST) kinases (MST1 and MST2) and large tumor suppressor (LATS) kinase (LATS1 and LATS2). Once Hippo pathway is activated, MST kinases would phosphorylate LATS kinases, phosphorylating the transcriptional co-activators yes-associated protein (YAP), thereby inactivating YAP [24–26]. YAP, as a major downstream effectors of Hippo pathway, has been reported to be increased and involved in the tumorigenesis of various cancers, such as liver cancer [27], breast cancer [28] and pancreatic cancer [29]. Previous studies have indicated that aberrant activation of YAP is one of the important mechanisms accounting for the pathogenesis of cancers [30]. The current study found that TANK1-ASODN induced an increase in phosphorylated YAP and a decrease of YAP. Overexpression of YAP could antagonize the repressive Hippo pathway through inhibiting the activity of LATS kinases [31]. Here, we found that TANK1-ASODN activated YAP through phosphorylation and inhibition of the canonical Hippo pathway kinase Last1. Besides, aberrant YAP overexpression has been implicated in fundamental cellular programs, such as cell proliferation, migration, invasion and EMT [32,33]. Considered together, TANK1-ASODN could inhibit cell proliferation, migration, invasion and EMT by regulating the expression of YAP via the Hippo pathway, thereby suppressing the progress of osteosarcoma.

5. Conclusion

Collectively, this study has shown that TANK1-ASODN could significantly inhibit *in vitro* MG-63 cell growth, migration, invasion and EMT through the Hippo/YAP signaling. The results in this study raised a novel strategy in the treatment of osteosarcoma and lay the groundwork for future research in cancer therapy.

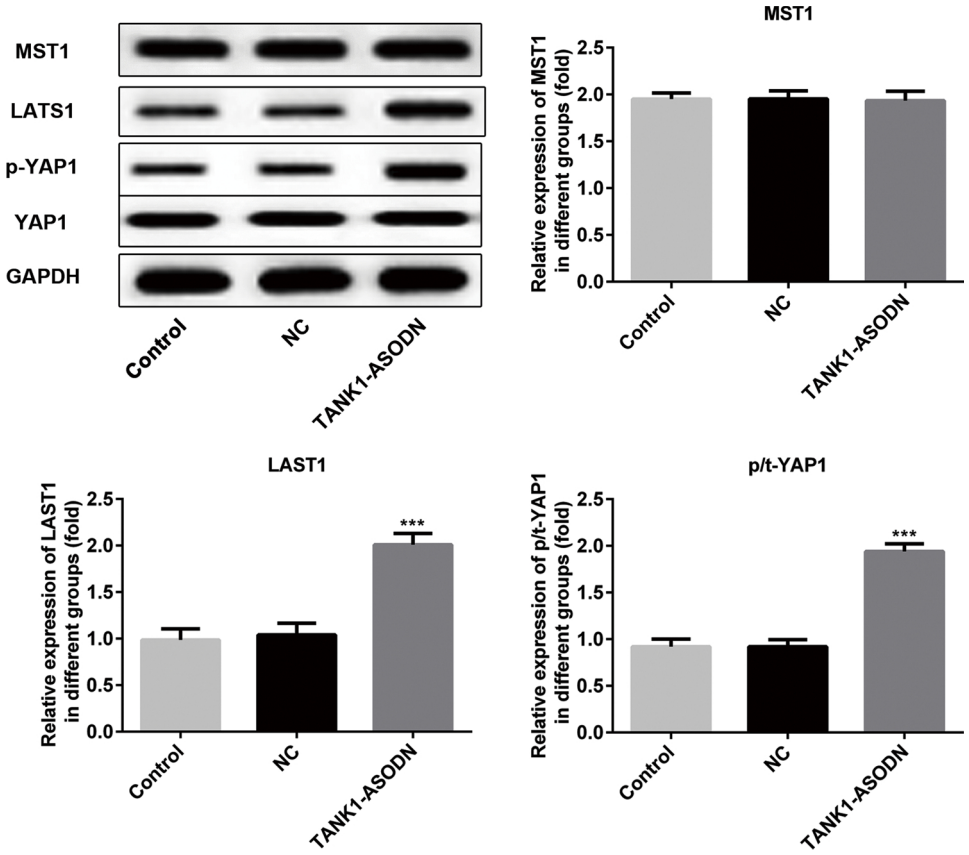

Fig. 5. TANK1-ASODN activated the Hippo/YAP signaling.
The relative protein expression of Hippo/YAP signaling was examined by western blot. The protein bands were quantified. Data is presented as the mean ± SD of three experiments. ***P < 0.001 vs control.

Conflict of interest

NO potential conflicts of interest were disclosed.

Acknowledgment

This project was funded by Health and Family Planning Commission of Hubei Province (WJ2018H1024).

References

[1] M.E. Anderson, Update on survival in Osteosarcoma, Orthop. Clin. North Am. 47 (1) (2016) 283–292.
[2] N.C. Daw, A.J. Chou, N. Jaffe, B.N. Rao, C.A. Billups, C. Rodriguez-Galindo, P.A. Meyers, W.W. Huh, Recurrent osteosarcoma with a single pulmonary metastasis: a multi-institutional review, Br. J. Cancer 112 (2) (2015) 278–282.
[3] C.M. Hattinger, M. Fanelli, E. Tavanti, S. Vella, S. Ferrari, P. Picci, M. Serra, Advances in emerging drugs for osteosarcoma, Expert Opin. Emerg. Drugs 20 (3) (2015) 495–514.
[4] A. Luetke, P.A. Meyers, I. Lewis, H. Juergens, Osteosarcoma treatment - where do we stand? A state of the art review, Cancer Treat. Rev. 40 (4) (2014) 523–532.
[5] C. Lengauer, K.W. Kinzler, B. Vogelstein, Genetic instability in colorectal cancers, Nature 386 (6625) (1997) 623–627.
[6] T. de Lange, Shelterin: the protein complex that shapes and safeguards human telomeres, Genes Dev. 19 (18) (2005) 2100-2010.
[7] R.K. Moyzis, J.M. Buckingham, L.S. Cram, M. Dani, L.L. Deaven, M.D. Jones, J. Meyne, R.L. Ratliff, J.R. Wu, A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes, Proc. Natl. Acad. Sci. U. S. A. 85 (18) (1988) 6622–6626.
[8] W. Palm, T. de Lange, How shelterin protects mammalian telomeres, Annu. Rev. Genet. 42 (2008) 301–334.
[9] C.B. Harley, A.B. Futcher, C.W. Greider, Telomeres shorten during ageing of human fibroblasts, Nature 345 (6274) (1990) 458–460.
[10] C.W. Greider, E.H. Blackburn, Identification of a specific telomere terminal transferase activity in Tetrahymena extracts, Cell 43 (2 Pt 1) (1985) 405–413.
[11] T.L.C. Li, Z. Zhan, Experimental research of tankyrase 1 antisense oligodeoxynucleotides on the proliferation of lung cancer cell nodules, Clin. Oncol. Cancer Res. 7 (3) (2010) 6.
[12] S.S. Hsiao SJ, Tankyrase function at telomeres, spindle poles, and beyond, Biochimie 90 (1) (2008) 10.
[13] G.I. Smith, S. Schmitt, A. de Lange, T. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres, Science 282 (5393) (1998) 4.
[14] J.R. Donigian, T. de Lange, The role of the poly(ADP-ribose) polymerase tankyrase1 in telomere length control by the TRF1 component of the shelterin complex, J. Biol. Chem. 282 (31) (2007) 22662–22667.
[15] S. Gelmini, M. Poggesi, V. Distante, S. Bianchi, L. Simi, M. Luconi, C.C. Raggi, L. Cataliotti, M. Pazzagli, C. Orlando, Tankyrase, a positive regulator of telomere elongation, is over expressed in human breast cancer, Cancer Lett. 216 (1) (2004) 81–87.
[16] S. Gelmini, M. Poggesi, P. Pinzani, S.C. Mannurita, F. Cianchi, R. Valanzano, C. Orlando, Distribution of Tankyrase-1 mRNA expression in colon cancer and its prospective correlation with progression stage, Oncol. Rep. 16 (6) (2006) 1261–1266.
[17] T.Y. Yeh, J.I. Sbodio, M.T. Nguyen, T.N. Meyer, R.M. Lee, N.W. Chi, Tankyrase-1 overexpression reduces genotoxin-induced cell death by inhibiting PARP1, Mol. Cell. Biochem. 276 (1-2) (2005) 183–192.
[18] N. Chi, L. Lehtiö, S. Krauss, Tankyrases as drug targets, FEBS J. 280 (15) (2013) 18.
[19] M.A. Cerone, N. McCabe, T. Ohishi, H. Seimiya, C.J. Lord, A. Ashworth, Targeting Tankyrase 1 as a therapeutic strategy for BRCA-associated cancer, Oncogene 28 (11) (2009) 6.
[20] C.A. de la Lastra, I. Villegas, S. Sanchez-Fidalgo, Poly(ADP-ribose) polymerase inhibitors: new pharmacological functions and potential clinical implications, Curr. Pharm. Des. 13 (9) (2007) 933–962.
[21] X. Song, M. Zhu, F. Zhang, F. Zhang, Y. Zhang, Y. Hu, L. Jiang, Y. Hao, S. Chen, Q. Zhu, W. Huang, J. Lu, J. Gu, W. Gong, M. Li, Y. Liu, ZFX promotes proliferation and metastasis of pancreatic Cancer cells via the MAPK pathway, Cell. Physiol. Biochem. 48 (1) (2018) 274–284.
[22] R.A. Mathias, S.K. Gopal, R.J. Simpson, Contribution of cells undergoing epithelial-mesenchymal transition to the tumour microenvironment, J. Proteomics 78 (2013) 545–557.
[23] B. Lv, X. Yang, S. Lv, L. Wang, K. Fan, R. Shi, F. Wang, H. Song, X. Ma, X. Tan, K. Xu, J. Xie, G. Wang, M. Feng, L. Zhang, CXCR4 signaling induced epithelial-mesenchymal transition by PI3K/AKT and ERK pathways in glioblastoma, Mol. Neurobiol. 52 (3) (2015) 1263–1268.
[24] B. Zhao, Q. Lei, K.L. Guan, Mst out and HCC in, Cancer Cell 16 (5) (2009) 363–364.
[25] Y. Bao, K. Nakagawa, Z. Yang, M. Ikeda, K. Withanage, M. Ishigami-Yuasa, Y. Okuno, S. Hata, H. Nishina, Y. Hata, A cell-based assay to screen stimulators of

the Hippo pathway reveals the inhibitory effect of dobutamine on the YAP-dependent gene transcription, J. Biochem. 150 (2) (2011) 199–208.
[26] X. Wei, B. Zhao, W. Li, R.S. Udan, Q. Yang, J. Kim, J. Xie, T. Ikenoue, J. Yu, L. Li, P. Zheng, K. Ye, A. Chinnaiyan, G. Halder, Z.C. Lai, K.L. Guan, Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control, Genes Dev. 21 (21) (2007) 15.
[27] L. Ma, J. Wang, W. Weng, Y. Qiao, Y. Zhang, J. He, H. Wang, W. Xiao, L. Li, Q. Chu, Q. Pan, Y. Yu, F. Sun, Mutual interaction between YAP and CREB promotes tumorigenesis in liver cancer, Hepatology 58 (3) (2013) 10.
[28] X. Zhang, X. Liu, J. Luo, W. Xiao, X. Ye, M. Chen, Y. Li, G.J. Zhang, Notch3 inhibits epithelial-mesenchymal transition by activating Kibra-mediated Hippo/YAP signaling in breast cancer epithelial cells, Oncogenesis 5 (11) (2016) e269.
[29] Z. Jiang, C. Zhou, L. Cheng, B. Yan, K. Chen, X. Chen, L. Zong, J. Lei, W. Duan, Q. Xu, X. Li, Z. Wang, Q. Ma, J. Ma, Inhibiting YAP expression suppresses pancreatic cancer progression by disrupting tumor-stromal interactions, J. Exp. Clin. Cancer Res. 37 (1) (2018) 69.
[30] U. Ehmer, J. Sage, Control of proliferation and Cancer growth by the hippo signaling pathway, Mol. Cancer Res. 14 (2) (2016) 127–140.
[31] U. Basu-Roy, N.S. Bayin, K. Rattanakorn, E. Han, D.G. Placantonakis, A. Mansukhani, C. Basilico, Sox2 antagonizes the Hippo pathway to maintain stemness in cancer cells, Nat. Commun. 6 (2015) 6411.
[32] S.W. Chan, C.J. Lim, K. Guo, C.P. Ng, I. Lee, W. Hunziker, Q. Zeng, W. Hong, A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells, Cancer Res. 68 (8) (2008) 2592–2598.
[33] H. Zhang, C.Y. Liu, Z.Y. Zha, B. Zhao, J. Yao, S. Zhao, Y. Xiong, Q.Y. Lei, K.L. Guan, TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition, J. Biol. Chem. 284 (20) (2009) 13355–13362.