Research Article

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Telmisartan induces osteosarcoma cells growth inhibition and apoptosis via suppressing mTOR pathway

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Abstract: Osteosarcoma (OS) is a commonly occurring primary malignant bone cancer with serious impact and high mortality, yet effective and safe therapy method not available. The aim of the present study was to elucidate the antitumor effect of telmisartan on human osteosarcoma cells in vitro and its underlying mechanism. The proliferation effect of osteosarcoma cell lines U2OS was examined by Cell Counting Kit-8. The invasive and migratory capabilities were determined by transwell invasion and migration assay. The percentage of apoptotic cells were detected by flow cytometric analysis and proteins related to apoptosis including Bax, Bcl-2 and Cleaved Caspase-3 were examined by western blotting. The expressions of mammalian target of rapamycin (mTOR) signaling relevant molecules were detected by western blot assay. Telmisartan treatment caused dose-dependent and time-dependent inhibition of proliferation and inducing anti-migration, anti-invasiveness and apoptosis of U2OS cells. The induction of apoptosis was confirmed concurring with the altered expression of proteins associated with the apoptosis. Mechanistically, telmisartan suppresses mTOR activation. Telmisartan can impede the growth, invasion, migration and induce the apoptosis of U2OS cell probably through inhibiting the mTOR signaling pathway activation. Thus, telmisartan is a potential drug for the prevention and treatment of human osteosarcomal cancer.

Keywords: Telmisartan, osteosarcoma, mTOR signaling, proliferation, apoptosis

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1 Introduction

Osteosarcoma (OS) is a common type of primary bone sarcoma in humans with high morbidity and mortality in both children and young adults, ordinarily presented as osteogenesis by malignant cells and characterized by high local aggressiveness and distant organic metastasize [1, 2]. In spite of the many advances in therapies, embodying surgical methods and neoadjuvant chemotherapy, surgery leading to limb amputation and chemotherapy as a common therapeutic method also brings forth multiple side effects, apart from that, a notable number of relapse or metastasis are still highly likely to occur [3-5]. Hence, the effective without deleterious OS therapies are still urgently needed.

Telmisartan is one of the angiotensin II type 1 receptor (AT1R) blockers (ARB). It has been well-known that AT1 and AT1R possess tumour-stimulative activities involved in motivating cell proliferation, migration, neo-angiogenesis and anti-apoptotis during cancer development [6-9], and ARBs inhibit these actions by antagonizing AT1R in various tumors [10-12]. Telmisartan as an ARB, is reported to have good antitumor activitiy including increased apoptosis of human prostate cancer cells [13], controlling gastric cancer cachexia [14], ameliorating autosomal recessive polycystic kidney disease [15], inhibiting tumor growth of esophageal adenocarcinoma [16], preventing proliferation and promoting apoptosis of human ovarian cancer [17] and so forth. However, there are no studies with telmisartan on OS yet. Most importantly, emerging report elaborated that risk of cancers including four most common cancers of lung, breast, prostate, and colorectal cancers are not increased with the use of telmisartan in comparison with other ARBs with large population-based study corroborating the results [18]. These evidence provides overwhelming support that telmisartan exerts a protective role in cancer treatment without adverse effect. Nonetheless, mechanisms of telmisartan mediated anticancer action are unclear yet, particulate in OS.

Thus, anticarcinogenic activity of telmisartan on OS and involved mechanisms are urgent to be explored.

In the present study, we demonstrate that telmisartan suppressed growth, invasiveness and migration and promoted apoptosis of OS cells. Furthermore, telmisartan treatment inhibited mTOR activation.

2 Materials and methods

2.1 Chemicals and antibodies

Telmisartan was purchased from MedChemExpress Biotechnology (Shanghai, China). Primary antibodies against the serine/threonine protein kinase B (AKT), phospho- (p-) AKT, mTOR, p-mTOR, B-cell lymphoma 2 (Bcl-2), Bcl-associated X (Bax), Cyclin D1, Cleaved Caspase-3, phospho-p70 ribosomal S6 kinase (p-P70S6K), Tubulin and anti-rabbit/mouse peroxidase conjugated secondary antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The enhanced chemiluminescence (ECL) detection system was from Proteintech Group, Inc (Wuhan, China).

2.2 Cell culture

Human OS cell lines (Shanghai Institute of Cell Biology, China) were grown in RPMI-1640 (Gibco Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), and penicillin-streptomycin sulfate (100 mg/mL; Invitrogen, Carlsbad, CA) at 37°C in a humidified atmosphere containing 5% CO_2. Cells in logarithmic growth phase (approximately 1×10^6 cells/ml) were used in the following experiment.

2.3 Assessment of Cell Proliferation

Cell proliferation was assayed using the Cell Counting Kit-8 (CCK-8) (Beijing solarbio science & technology co. Ltd., Beijing, China) according to the manufacturer's instructions. Briefly, 5×10^3 cells per well were seeded into 96-well plate and cultured in 100 μL of RPMI-1640 medium supplemented with 10% FBS for 24 h. Telmisartan (0, 1, 10, 20, 30, 40, 50 μM) was added to each well and cells were cultured for an additional 72 h, CCK-8 reagent (10 μL) was then added to each well, and the plates were incubated at 37°C for 1.5 h. The Optical density (OD) was measured at a wave-length of 450 nm using a microplate reader. Judging by results with concentration gradient, the action concentration of telmisartan treated in following experiment was identified, telmisartan at this concentration or vehicle (DMSO, 1‰ in culture media) was added to each well for 24 h, 48 h, 72 h. CCK-8 detection steps were the same as above. The following experimental group was treated with telmisartan at this concentration, and negative control group (NC) was cultured with 1‰ DMSO in culture media.

2.4 Cell migration and invasion assay

24-well transwell chamber with membrane pore size of 8.0 μm (Coring Costar, Cambridge, MA, USA) with or without Matrigel matrix (BD Sciences, San Jose, CA, USA) were used to assess cell invasive and migratory abilities, according to the protocol provided by the manufacture. In brief, Approximately 1×10^5 cells were suspended in 100 μl serum-free medium and plated in the upper chamber, while the lower chamber was filled with 500 μl of complete medium (medium containing 10% FBS). After incubation for 24 h at 37°C, 5% CO_2, non-invading cells on the top chamber were scraped off with cotton-tipped swabs, invaded cells were fixed with 4% paraformaldehyde for 30 min and then stained with 0.1% crystal violet for 20 min. Subsequently, the number of invaded cells was counted under a microscope in five random fields for each well.

For cell migration detection, the procedures resembled detection of cells invasion, but Matrigel was not applied.

2.5 Detection of apoptosis

Apoptotic U2OS cells were evaluated by an Annexin V-fluorescein isothiocyanate (Annexin V-FITC)/propidium iodide (PI) Apoptosis Detection kit (Beijing 4A Biotech Co. Ltd., Beijing, China) according to the manufacturer's instruction. The U2OS cells were treated with telmisartan and harvested by trypsinization without EDTA, washed twice with cold PBS, centrifuged at 3000 r/min for 5 min and the supernatant was discarded, the pellet was resuspended in 500 μl 1 × binding buffer. Then, 5 μl FITC-conjugated Annexin V and 10 μl PI were added. After incubation for 5 min at room temperature in the dark, the cells were analyzed with a FACScan instrument (FACS Calibur, BD Biosciences, CA).

2.6 Western blot analysis

Total proteins were isolated from cells with RIPA lysis buffer (cwbiotech, Beijing, China). After lysis for 30 min on ice, the lysates were centrifuged at 12,000 rpm at 4°C for 15 min. Total protein concentration was determined by BCA assay (cwbiotech, Beijing, China). Equal amounts of total protein was loaded and separated on 8%-10% Tris-glycine gradient gels via dodecyl sulfate-polyacrylamide gel electrophoresis. Subsequently, the proteins were transferred to poly vinylidene difluoride membranes for 2 h and blocked with 5% non-fat milk in TBST buffer (pH7.4 tris-buffered saline buffer containing 0.1% Tween-20) for 1.5 h. Then, the membranes were incubated with primary antibodies (1 : 1000 dilution) against AKT, p-AKT, mTOR, p-mTOR, Cyclin D1, p-P70S6K, Bcl-2, Bax, Cleaved Caspase-3, and against Tubulin (1 : 10000 dilution) at 4°C overnight, and were detected using anti-rabbit/mouse horseradish peroxidase -conjugated secondary antibodies (1 : 5000 dilution). Western blot bands were detected by ECL kit and were measured with Image-Pro Plus6.0 software.

2.7 Statistical analysis

The Student's t-test was utilized to determine significant differences. SPSS (International Business Machines Corporation, New York, USA) 22.0 software and GraphPad Prism 5 (San Diego, CA, USA) were used to perform statistical analysis. Images were quantified by Image-Pro Plus6.0 software (Media Cybernetics, Maryland, USA). All values were expressed as the mean ± standard deviation (SD) and evaluated by an analysis of Student's t-test. The differences between two groups were considered to be significant at a $^{**}p < 0.01$. All assays were conducted in triplicate.

3 Results

3.1 Effect of telmisartan on the proliferation of OS cell lines in vitro

To investigate the influence of telmisartan on OS cell proliferation, CCK8 was used to analyze cell viability in vitro. Firstly, we detected cell viability treated U2OS cells with telmisartan at 0, 1, 10, 20, 30, 40, 50 μM and observed that the cell viability was dose-dependent with telmisartan treatment, but 30 μM and above concentration, the cell viability was less than 50% when compared with 0 μM (Fig. 1A), which was not conducive to the following experiment. Therefore, we selected 20 μM as the candidate concentration. Next, we found that antiproliferative abilities in U2OS cells induced by telmisartan presented time-dependent, and especially at 72 h timepoint, this proliferation-inhibitory effect was more evident than other timepoint (Fig. 1B, $^{**}p < 0.01$).

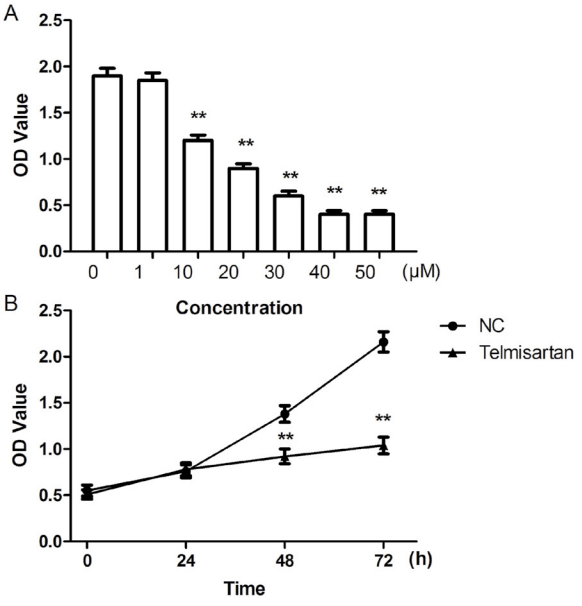

Figure 1. The effect of telmisartan on the proliferation of OS cell lines in vitro by CCK8 assay. A. Dose-dependent effect of telmisartan on the viability of OS cells. $^{**}p < 0.01$ versus 0 μM groups. B. Time-dependent effect of telmisartan on the viability of OS cells. Values are presented as mean ± SD. $^{**}p < 0.01$ versus NC groups.

3.2 Effect of telmisartan on migration and invasion of OS cells

Following, transwell invasion and migration assay were performed to determine cell invasive and migratory capabilities. As shown in Fig. 2A, after treatment with telmisartan, the numbers of invaded cells were markedly decreased to 25 ± 2 compared with the control (39 ± 4) ($^{**}p < 0.01$). Likewise, result of transwell migration assay showed that telmisartan treatment dramatically reduced migrated cells numbers (63 ± 5), comparable to negative control (NC) groups (82 ± 8) (Fig. 2B, $^{**}p < 0.01$).

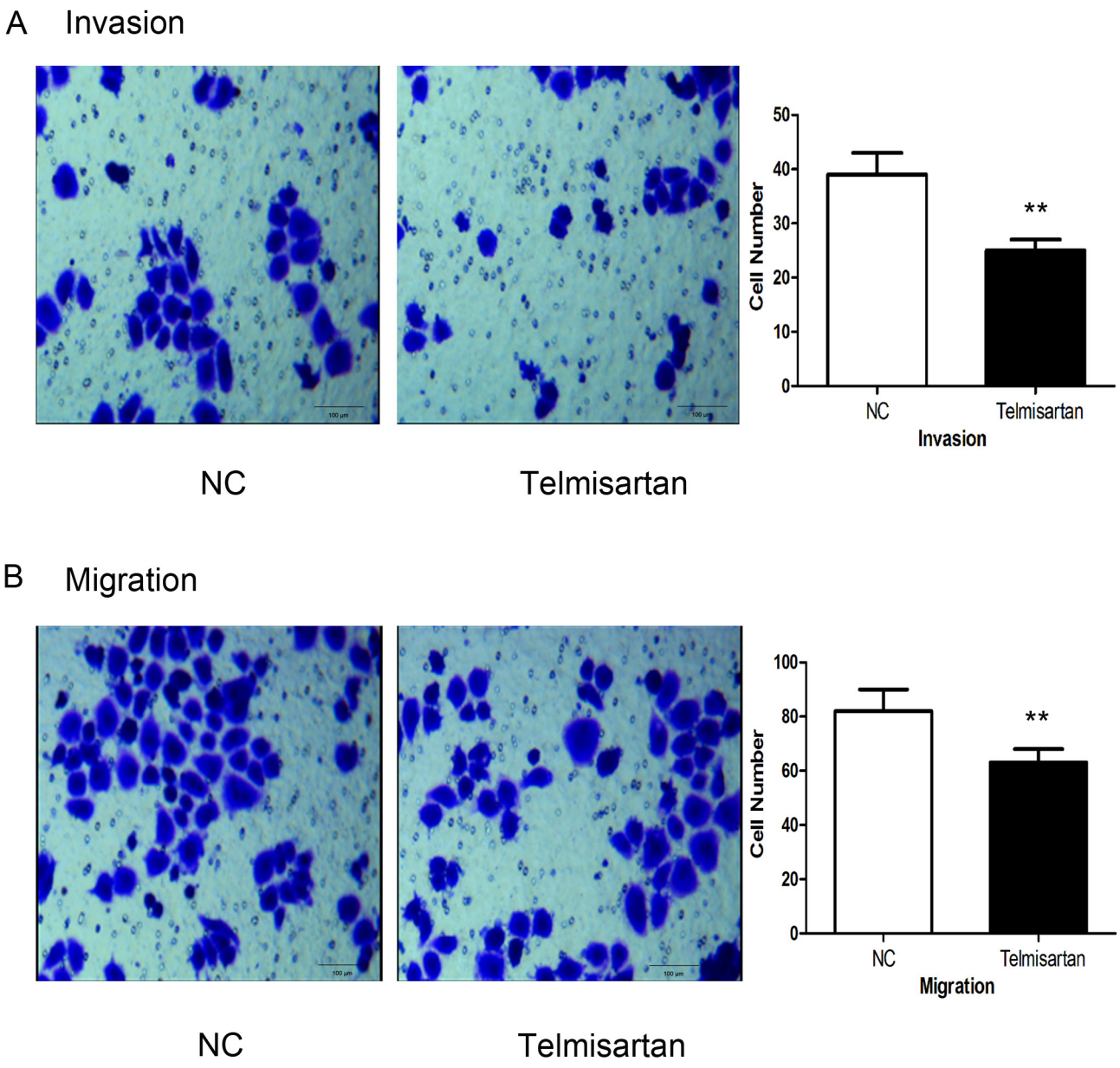

Figure 2. The change of invasive and migratory capabalities in OS cell lines after telmisartan treatment were assessed by Transwell membrane migration and invasion assays. A. Telmisartan obviously reduced the number of cells invaded to the chamber. B. Telmisartan also markedly reduced the number of cells migrated to the chamber. $^{**}p < 0.01$ versus NC groups.

3.3 Apoptotic changes in the OS cells treated with telmisartan

To ascertain whether OS cell death induced by telmisartan was presented through apoptosis, cells were treated with telmisartan and then stained with Annexin-V-FITC/ PI. We found that telmisartan administration of U2OS cells results in a markedly increase in apoptotic rate of treated groups (24.37% ± 0.92%) comparable to NC groups (6.13% ± 0.43%) (Fig. 3A). To study the mechanism mediating telmisartan-induced apoptosis, Bax, Bcl-2, Cleaved Caspase-3 activities were examined in telmisartan-administrated cells using western blotting and results discovered suggest that the expression of the anti-apoptotic protein, Bcl-2, was reduced while the pro-apoptotic protein Bax was increased significantly (Fig. 3B and C, $^{**}p < 0.01$). Meanwhile, the expression level of Cleaved Caspase-3 was also upregulated in telmisartan-treated groups ($^{**}p < 0.01$).

3.4 The effects of treatment with telmisartan on mTOR signaling activity

It has been demonstrated that mTOR signaling plays a crucial cancer-inhibitory role in a variety of tumors. Thus, to determine the role of telmisartan in the activation of mTOR signaling, we detected series of correlative molecules with this vital pathway. As shown in Fig. 4A and B, telmisartan treatment leads to significant reduction in the phosphorylation levels of AKT and mTOR, while the total AKT, and mTOR protein level were observed without substantial change. Besides, telmisartan administration attenuated activity of mTOR downstream molecules such as p-P70S6K and Cyclin D1 related to cell growth. Together, these results showed that telmisartan inhibited the activation of mTOR signaling pathway.

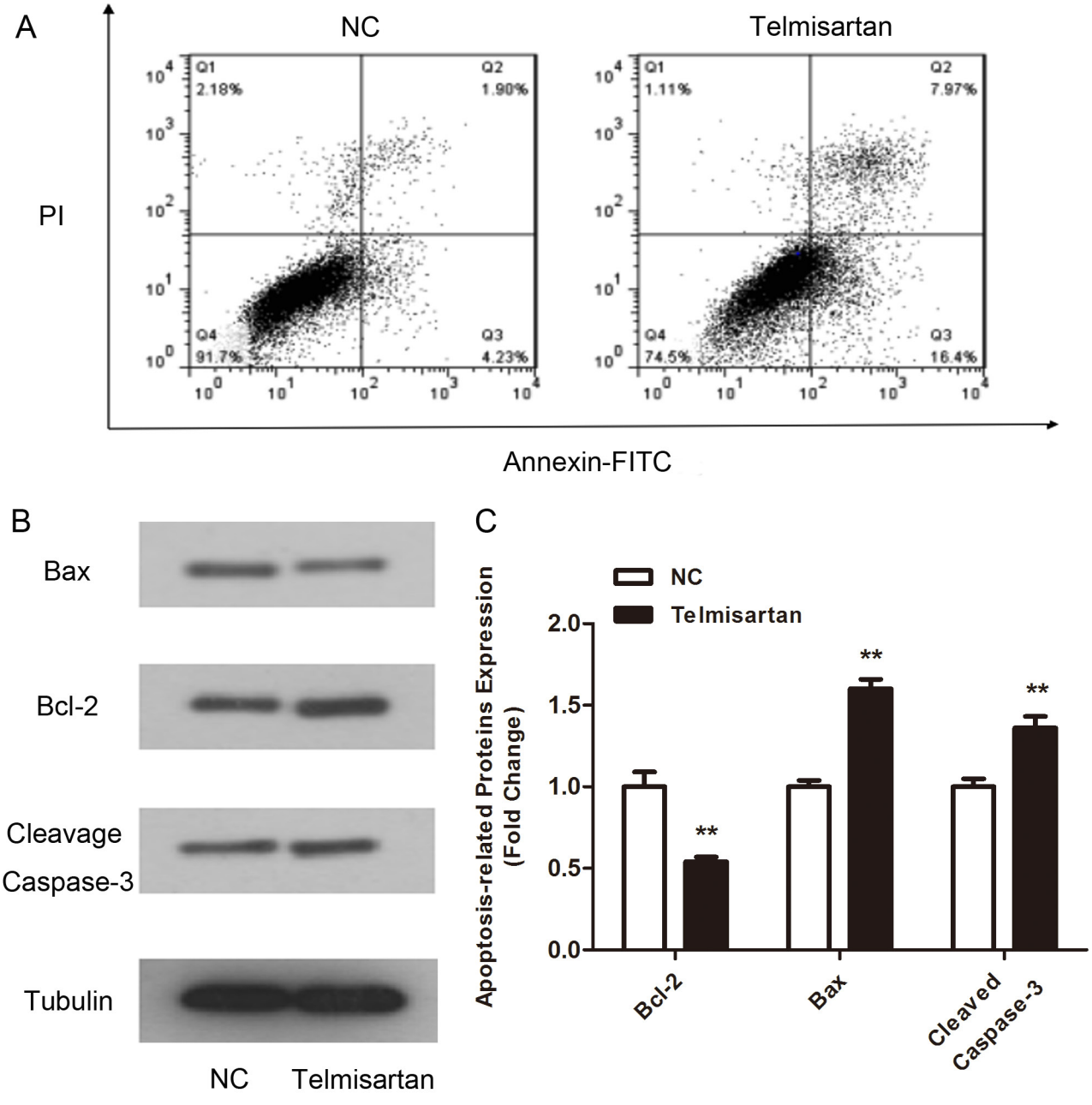

Figure 3. Inductive impact of apoptosis by telmisartan in OS cells. A. The percentage of apoptotic cells were observed after stained with Annexin-FITC/PI and detection of flow cytometry to be significantly increase by administration of telmisartan. B. Expression of the apoptosis-related proteins including Bax, Bcl-2 and Cleaved Caspase-3 treated with telmisartan in OS cells were determined by western blot analysis. C. Quantified results that analyzed by Image-Pro Plus6.0 software were presented with histogram types. All values are expressed as mean ± SD. $^{**}p < 0.01$ versus NC groups.

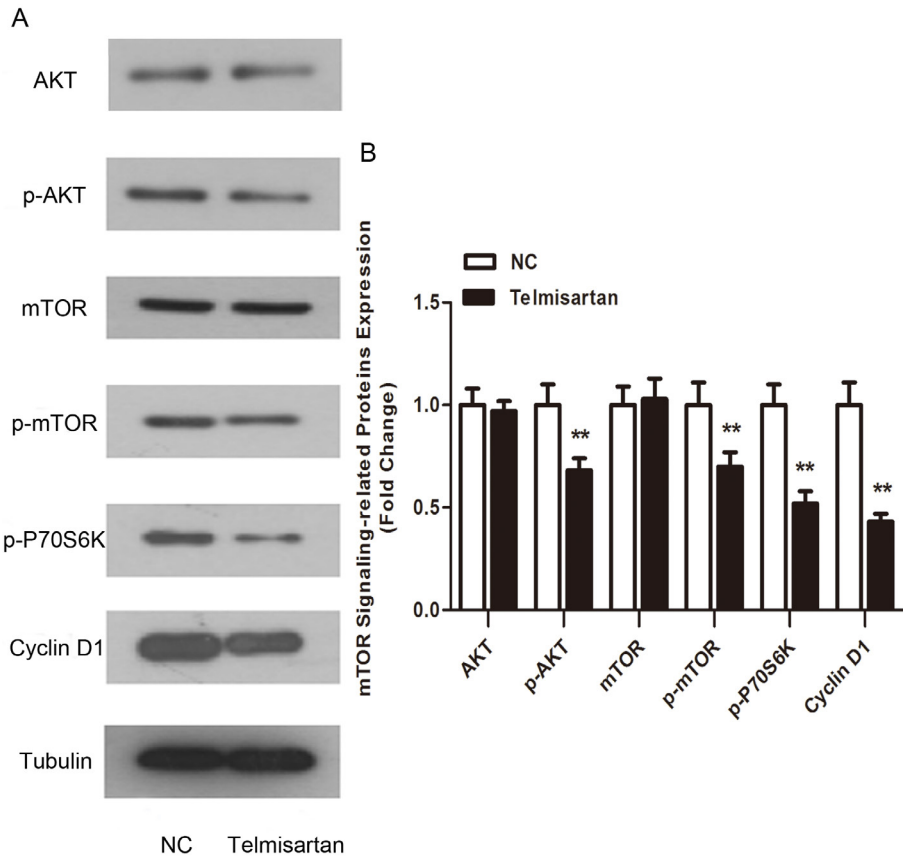

Figure 4. The impact of mTOR activity by telmisartan treated in OS cells. A. The expression of crucial proteins related to mTOR signaling, namely, AKT, p-AKT, mTOR, p-mTOR, p-P70S6K, Cyclin D1 were detected by western blot analysis. B. Quantified data after analyzed by Image-Pro Plus6.0 software were presented with histogram types. All values are expressed as mean ± SD. $^{**}p < 0.01$ versus NC groups.

4 Discussion

In this study, our present finding indicated that the effect of telmisartan on the growth of OS cell lines and relevant mechanism of action. Above all, we found telmisartan markedly inhibited OS cells proliferation, migration and invasion. Further, we discovered that telmisartan induced apoptosis of OS cells. Moreover, we revealed that the above antitumorigenic potent effects of telmisartan might be regulated by inhibition of the mTOR pathway.

Accumulating evidence shows that telmisartan takes on a critical anticarcinogenic action in various types of cancers via in vitro and in vivo experiments. For example, a current study showed that telmisartan effectively suppressed abdominal aortic aneurysms pathogenesis [19]. Another research reported that telmisartan has antiproliferative and apoptotic effects on human colon cancer in vitro [20]. In concert with these findings, in present study, we found that telmisartan is able to down-regulate growth, invasion and migration in OS cell lines obviously. Additionally, administration of OS with telmisartan could induce apoptosis occurrence. Previous studies have shown that some of the cancerous cell proliferation inhibitory action was contributed by apoptosis [21, 22]. As is known that Bcl-2, Bax and Cleaved Caspase-3 play crucial role in apoptosis process [23]. Thus, in our study, OS cells apoptotic ratio was also incremented after telmisartan treatment, further, this induction was accompanied by a strong decrement in anti-apoptotic protein Bcl-2 expression level, while a high increase in pro-apoptosis proteins Bax and Cleaved Caspase-3. Above of these data indicate that telmisartan has a tumor suppressor potential in OS. In this case, series of anti-tumor potencies with telmisartan treatment in OS were presented, which prompt us to explore its underlying mechanism.

To investigate relevant molecular mechanism, we assess activity of mTOR signaling pathway. It has been well-established that mTOR pathway is involved in cell survival, growth and metastasis, and is one of the major signaling

pathways tightly correlated with cancer progression. mTOR pathway is reported to be activated in osteosarcoma cell lines [24, 25]. Once PI3K activated, its catalytic subunit activates AKT, successively mTOR complex 1 (mTORC1) is activated, and subsequently activation of mTORC1 upregulates P70S6K phosphorylation and then control protein synthesis like Cyclin D1, consequently modulating cellular growth and apoptosis process [26, 27]. Increasing reports demonstrated that mTOR pathway mediated tumorigenicity in OS [28-30]. What is more, there is some reports mentioned that telmisartan participated in regulation of mTOR pathway [16, 31]. In the current study, we evaluated mTOR signaling correlative proteins and found that telmisartan led to reduction of p-mTOR, p-AKT expression levels, and also discovered that expression of Cyclin D1 and p-P70S6K were suppressed by telmisartan treated. Collectively, these results indicated that mTOR signaling pathway was mediated human OS cell potent anti-proliferative, anti-metastatic, and pro-apoptotic effects. However, the limitation of our study were that we merely apply single OS cell line U2OS, and confined to study in cellular level. Thereby, further studies with different cell lines or in vivo and more elaborate molecular mechanism related pathway or others might be needed to investigate.

In conclusion, the present results manifest that telmisartan has anticancer function in human OS cells through inhibiting the potency of cell proliferation, invasion, migration and enhancing apoptosis of human OS cells. In addition, telmisartan develops its potential action in resisting OS cells probably through mTOR inactivation. Based on our findings, telmisartan could be considered as a promising novel therapeutic candidate for OS.

Ethical approval: The conducted research is not related to either human or animals use.

Conflict of interest: Authors state no conflict of interest.

Abbreviations

OS	osteosarcoma
mTOR	mammalian target of rapamycin
AT1R	angiotensin II type 1 receptor
ARB	angiotensin II type 1 receptor blockers
AKT	serine/threonine protein kinase B
Bcl-2	B-cell lymphoma 2
Bax	Bcl-associated X
p-P70S6K	phospho-p70 ribosomal S6 kinase
ECL	enhanced chemiluminescence
CCK-8	Cell Counting Kit-8
Annexin V-FITC /PI	Annexin V-fluorescein isothiocyanate/ propidium iodide

References

[1] Arndt CA, Rose PS, Folpe AL, Laack NN. Common musculoskeletal tumors of childhood and adolescence. Mayo Clin. Proc. 2012; 87:475-487.
[2] Yu W, Zhu J, Wang Y, Wang J, Fang W, Xia K, et al. A review and outlook in the treatment of osteosarcoma and other deep tumors with photodynamic therapy: from basic to deep. Oncotarget. 2017; 8:39833-39848.
[3] L A, MA A, DL M, GL F. Survival, Recurrence, and Function After Epiphyseal Preservation and Allograft Reconstruction in Osteosarcoma of the Knee. Clinical Orthopaedics and Related Research. 2015; 473:1-5.
[4] Anderson ME. Update on Survival in Osteosarcoma. Orthop. Clin. North Am. 2016; 47:283.
[5] Canter RJ. Chemotherapy: Does Neoadjuvant or Adjuvant Therapy Improve Outcomes? Surg. Oncol. Clin. N. Am. 2016; 25:861-872.
[6] Li SH, Lu HI, Chang AY, Huang WT, Lin WC, Lee CC, et al. Angiotensin II type I receptor (AT1R) is an independent prognosticator of esophageal squamous cell carcinoma and promotes cells proliferation via mTOR activation. Oncotarget. 2016; 7:67150-67165.
[7] Dolley-Hitze T, Verhoest G, Jouan F, Le PP, Arlot-Bonnemains Y, Oger E, et al. [Angiotensin-2 type 1 receptors (AT1R) and cancers]. Nephrol. Ther. 2013; 9:85-91.
[8] Huang MM, Guo AB, Sun JF, Chen XL, Yin ZY. Angiotensin II promotes the progression of human gastric cancer. Mol. Med. Report. 2014; 9:1056-1060.
[9] Itinteang T, Marsh R, Davis PF, Tan ST. Angiotensin II causes cellular proliferation in infantile haemangioma via angiotensin II receptor 2 activation. J. Clin. Pathol. 2015; 68:346-350.
[10] Shen J, Huang YM, Wang M, Hong XZ, Song XN, Zou X, et al. Renin-angiotensin system blockade for the risk of cancer and death. Journal of the Renin-Angiotensin-Aldosterone System,17,3(2016-7-01). 2016; 17:pii: 1470320316656679.
[11] Okazaki M, Fushida S, Harada S, Tsukada T, Kinoshita J, Oyama K, et al. The Angiotensin II type 1 receptor blocker candesartan suppresses proliferation and fibrosis in gastric cancer. Cancer Lett. 2014; 355:46-53.
[12] Kim S, Toyokawa H, Yamao J, Satoi S, Yanagimoto H, Yamamoto T, et al. Antitumor effect of angiotensin II type 1 receptor blocker losartan for orthotopic rat pancreatic adenocarcinoma. Pancreas. 2014; 43:886-890.
[13] Wu F, Zhou L, Jin W, Yang W, Wang Y, Yan B, et al. Anti-Proliferative and Apoptosis-Inducing Effect of Theabrownin against Non-small Cell Lung Adenocarcinoma A549 Cells. Frontiers in pharmacology. 2016; 7:1-10.
[14] Sukumaran S, Patel HJ, Patel BM. Evaluation of role of telmisartan in combination with 5-fluorouracil in gastric cancer cachexia. Life Sci. 2016; 154:15-23.
[15] Yoshihara D, Kugita M, Mai S, Horie S, Nakanishi K, Abe T, et al. Telmisartan Ameliorates Fibrocystic Liver Disease in

an Orthologous Rat Model of Human Autosomal Recessive Polycystic Kidney Disease. PLoS One. 2013; 8:e81480.
[16] Fujihara S, Morishita A, Ogawa K, Tadokoro T, Chiyo T, Kato K,, et al. The angiotensin II type 1 receptor antagonist telmisartan inhibits cell proliferation and tumor growth of esophageal adenocarcinoma via the AMPKa/mTOR pathway in vitro and in vivo. Oncotarget. 2017; 8:8536-8549.
[17].Pu Z, Zhu M, Kong F. Telmisartan prevents proliferation and promotes apoptosis of human ovarian cancer cells through upregulating PPARγ and downregulating MMP-9 expression. Mol. Med. Report. 2016; 13:555-559.
[18] Tascilar K, Azoulay L, Dell'Aniello S, Bartels DB, Suissa S. The Use of Telmisartan and the Incidence of Cancer. Am. J. Hypertens. 2016; 29:1358-1365.
[19] Xuan H, Xu B, Wang W, Tanaka H, Fujimura N, Miyata M, et al. Inhibition or deletion of angiotensin II type 1 receptor suppresses elastase-induced experimental abdominal aortic aneurysms. J. Vasc. Surg. 2018; 67:573-584.
[20] Lee LD, Mafura B, Lauscher JC, Seeliger H, Kreis ME, Gröne J. Antiproliferative and apoptotic effects of telmisartan in human colon cancer cells. Oncol. Lett. 2014; 8:2681-2686.
[21] Matsuyama M, Funao K, Kuratsukuri K, Tanaka T, Kawahito Y, Sano H, et al. Telmisartan inhibits human urological cancer cell growth through early apoptosis. Exp. Ther. Med. 2010; 1:301-306.
[22] Cheriyan VT, Wang Y, Muthu M, Jamal S, Chen D, Yang H, et al. Disulfiram Suppresses Growth of the Malignant Pleural Mesothelioma Cells in Part by Inducing Apoptosis. PLoS One. 2014; 9:e93711.
[23] Rf DAJ, Leitão Oliveira AL, Rf DMS, Ha DOR, De FCP, de Araújo AA. Telmisartan induces apoptosis and regulates Bcl-2 in human renal cancer cells. Exp. Biol. Med. 2015; 240:34-44.
[24] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012; 149:274.
[25] Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR Signaling in Cancer. Front. Oncol. 2014; 4:1-11.
[26] Fasolo A, Sessa C. mTOR inhibitors in the treatment of cancer. Expert Opinion on Investigational Drugs. 2008; 17:1717-1734.
[27] Chen W, Luo Y, Liu L, Zhou H, Xu B, Han X, et al. Cryptotanshinone inhibits cancer cell proliferation by suppressing mTOR-mediated cyclin D1 expression and Rb phosphorylation. Cancer Prevention Research. 2010; 3:1015-1025.
[28] Ding L, Congwei L, Bei Q, Tao Y, Ruiguo W, Heze Y, et al. mTOR: An attractive therapeutic target for osteosarcoma? Oncotarget. 2016; 7:50805-50813.
[29] Gao S, Sun H, Cheng C, Wang G. BRCA1-Associated Protein-1 Suppresses Osteosarcoma Cell Proliferation and Migration Through Regulation PI3K/Akt Pathway. DNA Cell Biol. 2017; 36:386-393.
[30] Jiang H, Wang X, Miao W, Wang B, Qiu Y. CXCL8 promotes the invasion of human osteosarcoma cells by regulation of PI3K/Akt signaling pathway. APMIS. 2017; 125:773-780.
[31] Xu L, Liu Y. Administration of telmisartan reduced systolic blood pressure and oxidative stress probably through the activation of PI3K/Akt/eNOS pathway and NO release in spontaneously hypertensive rats. Physiol. Res. 2013; 62:351-359.