European Review for Medical and Pharmacological Sciences 2019; 23 (3 Suppl): 81-88

TAZ accelerates osteogenesis differentiation of mesenchymal stem cells via targeting PI3K/Akt

F.-Z. TAN[1], H.-L. DAI[2]

[1]Department of Orthopedics, First Affiliated Hospital of Heilongjiang University of Chines Medicine, Harbin, China
[2]Department of Urinary Surgery, Dongfang Hospital of Beijing University of Chi Medicine, Beijing, China

Abstract. – OBJECTIVE: To investigate the promoting effects of TAZ on the osteogenic differentiation of mesenchymal stem cells in a rat model of osteoporosis through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signal.

MATERIALS AND METHODS: A total of 12 Sprague-Dawley (SD) rats were randomly divided into normal group (n=6) and model group (n=6). The rats in normal group were not given any treatment, while those in model group were used to establish a model of osteoporosis, and the bone mineral density (BMD) of the fe and lumbar in each group was detected. the bone marrow mesenchymal stem cells observed, of which those in normal group v divided into normal group and inhibitor gro The bone marrow mesenchyma cells model group were enrolled as oup. Al kaline phosphatase (ALP) ing v adopted to observe the osteo sis, ce counting kit-8 (CCK-8) was applied ete proliferation, and i mistry w performed to mea the ex ion of TAZ. Western blottin utilized to t the relative expressio sphorylated (p-Akt) and bone mo nogen protein 2 (BMP-2) proteins, and uantitative merase Chain Reaction (qP) was used to sure the messenger ri ucleic acid (mRNA) pression of TAZ.

RESULTS: e average optical density of A positi ells was decreased remarkably in m oup and hibitor group compared with t n norm group ($p<0.05$), while the ll pro tio e was increased notably in del gro inhibitor group. In comparison h normal oup, model group and inhibitor d evidently declined positive expression RNA expression of TAZ ($p<0.05$). The relative expressions of p-Akt and BMP-2 proteins in model group and inhibitor group were nificantly lower than those in normal group ($p<0.05$).

CONCLUSIONS: TAZ promotes the osteogenic differentiation of mesenchymal stem cells in the rat model of osteoporosis by repressing the PI3K/Akt signal.

Key Words:
Osteoporosis, T PI3K/Akt s ng nway, Bone marrow m mal stem cel

Introdu n

teoporosis is a systemic disease in which ra d bone fragi due to osteopenia, as well as de d bone de y and mass, increases the risk of f e[1,2]. A common disease among the elderly, osis often leads to fracture and ny secondary complications, thus threatening d quality of life. According to statistics, e ost oporosis-induced disabling injuries have become the third most serious diseases following hypertension, cardiovascular, and cerebrovascular diseases that affect the life and health of the elderly[3]. It is currently believed that proliferation and differentiation of bone marrow mesenchymal stem cells, especially the osteogenic differentiation, have close correlations with the onset and recovery of osteoporosis, providing new ideas for the treatment and study of the disease.

As an important cell signal transduction pathway, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway plays a vital role in mediating numerous processes, including cell proliferation, differentiation, apoptosis, and necrosis[4]. Currently, it is argued that the PI3K/Akt signaling pathway can accelerate the proliferation and differentiation of pre-osteoblastic cells, Regulation on this pathway is conducive to decreasing the proliferation of bone marrow mesenchymal stem cells and promoting the differentiation into osteoblasts.

TAZ, i.e., a transcriptional coactivator with PDZ-binding motif, has been proven to exert important effects in such processes as cell proliferation, differentiation, and self-renewal. It is not only a crucial transcription factor, but also

Corresponding Author: Hongliang Dai, BM; e-mail: daihl973211@sina.com

an important regulatory nuclear factor of the Hippo signaling pathway[5,6]. This research aims to explore whether TAZ promotes the osteogenic differentiation of mesenchymal stem cells in the rat model of osteoporosis through the PI3K/AKT signal. Also, it aims to further clarify its mechanism of action.

Materials and Methods

Laboratory Animals

A total of 12 female SPF Sprague-Dawley (SD) experimental rats (aged 1-month-old) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. [license number: SCXK (Shanghai, China) 2014-0003]. These rats were fed in the Laboratory Animal Center with normal diet and sterile filtered water every day under the conditions of a 12/12 h light/dark cycle, conventional room temperature, and humidity. This research was approved by the Animal Ethics Committee of the Heilongjiang University of Chinese Medicine.

Experimental Reagents and Instrum

L-glutamine (Gibco, Rockville, MD, U), Dulbecco's Modified Eagle's Medium (DM Gibco, Rockville, MD, USA), fetal bovine rum (FBS; Gibco, Rockville, M SA). Pr mary antibodies: anti-TAZ Abcam, Cambridge, MA, USA), u-phos orylated Akt (p-Akt) antibody (Ab Cam MA, USA), anti-runt-relat tra (RUNX2) antibod bcam, bridge, MA, USA), anti-bone hogenetic p 2 (BMP-2) antibody (Cambridge, USA), secondary ant odies am, Cambridge, MA, USA), al ne phospha (ALP) staining kit, enzyn nked immunoso assay (ELISA) kit eQ qua ative polymerase chain reaction (q). SY Green Master Mix kit (Vazyme, Nanj na), HiS pt II Q RT SuperMix for qPCR DNA r) kit (Vazyme, Nanjing, ina), li scope (Leica DMI 4000B/DFC, Mü n, Germany), fluorescence qPCR ment (ABI 7500; Applied Biosystems, Fos- , USA).

Animal Grouping and Treatment

All the 12 female SD rats were divided into normal group (n=6) and model group (n=6) using a random number table. The rats were fed adaptively in the Laboratory Animal Center for 7 d for subsequent experiments.

The rats in normal group were fed normally without any treatment. Their bone marrow mesenchymal stem cells were taken for *in vitro* culture. The rats in model group were utilized to establish an osteoporosis model and the bone marrow mesenchymal stem cells were t for *in vitro* culture after successful model

Establishment of Osteopor is Mo

The rats were anesthetized intraperit injection of 2% pentobarbit odium 2 mL/k and then fixed on the o ion ta in supine position. Next, an abdom ine incision was made, the junct of ovi and uter was ligated, and the o was excis fte t, the wound was w nd sutured by layer. At 12 week ter t peration, the bone mineral density (BMD) w easured to determine whet model was es hed successfully.

D ection of BMD

ter success anesthesia, the rats in each gr were fixe n a dual-energy X-ray absorp ter to ect, analyze, and compare the BMD o and femur to confirm the successful modeling.

In Vitro Culture and Grouped Treatment of Bone Marrow Mesenchymal Stem Cells

Once the rats in each group were anesthetized, the femur and tibia were exposed rapidly and the articular surfaces and metaphyses at both ends of the long bone were cut open to expose the medullary cavity. Then, the bone marrow in the medullary cavity was drawn to harvest the bone marrow mesenchymal stem cells, followed by pipetting and centrifugation. The harvested bone marrow mesenchymal stem cells were added into the prepared medium for resuspension, seeded into a culture dish at a density of 10^5/mL, and placed in a cell incubator for culture. The medium was replaced every 3 d, and the culture was continued after passage. The cells in the third passage were used for experiments.

The bone marrow mesenchymal stem cells from the rats in normal group were divided into normal group and inhibitor group, while those from the rat model of osteoporosis were enrolled into model group. Osteogenic induction: the cells were seeded into a 6-well plate at a density of 10^5cells/well for culture, and osteogenic induction was conducted after cell fusion. The osteogenic induction medium was directly added into the

cells in normal group and model group, and the cells were collected for detection after 3 days of induction. In inhibitor group, the cells were processed with 1 μmol/L inhibitor LY294002 for 24 h first. Then, the osteogenic induction medium was added for induction for 3 d, after which the cells were collected for detection.

Immunohistochemistry and ALP Staining

After the completion of the intervention, the culture medium was removed and the cells were fixed with 4% paraformaldehyde for 4 h. Next, endogenous peroxidase blocker was added dropwise onto the specimens and reacted for 10 min after rinsing. Subsequently, the specimens were rinsed and added with goat serum for sealing for 20 min. The anti-TAZ primary antibody (1:200) was added after the goat serum blocking buffer was shaken off, followed by culture in a refrigerator at 4°C overnight. On the next day, the specimens were rinsed and added with the secondary antibody solution for a reaction for 10 min. After rinsing adequately, the streptavidin-peroxidase solution was added for a reaction for 10 min, followed by color development with diaminobenzidine (DAB) a in drops, counterstaining of the nucleus wit matoxylin, mounting, and observation.

At 7 d after the osteogenic induction, the c ture medium was removed, and ells we fixed with fixative at room tem atu r 1 mi after rinsing. The staining ion wa repared in accordance with the kit uctio into the 6-well plate in ops, at room temperatur X h an ervation. After rising, the cel e digested w rypsin and harvested, foll entrifugation 2,000 g for 5 min and ysis. L the ALP staining was quantifie ing p-nitrop phosphate method and th erage optical den t 450 nm in each wel as mea d.

Detection of Cell Proliferation Via Cell Counting Kit-8 (CCK-8) Assay

The ce ch group were seeded in a well plat a density of 10^5 cells/well. The ls in normal group and model group were c rmally without any treatment, while those in inhibitor group were cultured in a medium containing 1 μmol/L inhibitor LY294002. ter culturing for 24 h, the CCK-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was added to each group, and the cell proliferation rate in each group was detected according to the CCK-8 instructions.

Western Blotting

The lysis buffer was added into the cells for an ice bath for 1 h. Then, the cells were put into a centrifuge for centrifugation at 14,000 g for 10 min, and the protein was quantified usin BCA method. Next, the absorbance and dard curve of the protein were obtained thr a microplate reader, which were applied t culate the protein concentration in tissue Subse tly, the proteins in tissue specimen re subject denaturation and sodium d yl sul ate-po acrylamide gel electroph s (SDS GE) separation. The position of th proteins as observed and the trophe was s ped when the Mark roteins rea th ottom of the glass a straight l hen, the proteins we trans d onto a polyvinylidene difluoride (PVDF) me ne (Millipore, Billerica, M A) and react ith blocking buffer for h. After that, anti-p-A t primary antibody (1 00), anti-RUNX2 primary antibody (1:1000), a BMP-2 prim antibody, and secondary antib (1:1000) e added in sequence. Later, the p ins w subjected to sufficient color develop h the chemiluminescent reagent the dark for 1 min after rinsing.

Detection Via QPCR

The knee joint tissue stored for standby use was added into the ribonucleic acid (RNA) extraction reagent to extract the total RNA in the specimens. Then, the extracted total RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using the reverse transcription kit, with a reaction system of 20 μL. Reaction conditions are as follows: reaction at 53°C for 5 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 10 s, and annealing at 62°C for 30 s for 35 cycles. ΔCt was calculated first, and then the expression difference of target genes was calculated. The detailed primer sequences are shown in Table I.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was adopted for statistical analysis. The enumeration data were expressed as mean ± standard deviation. Comparison between multiple groups was done using One-way ANOVA test followed by Post Hoc Test (Least Significant Difference). *t*-test was performed for data meeting normal distribution and homogeneity of variance, corrected *t*-test for those meeting normal distribution and

Table I. Primer sequences.

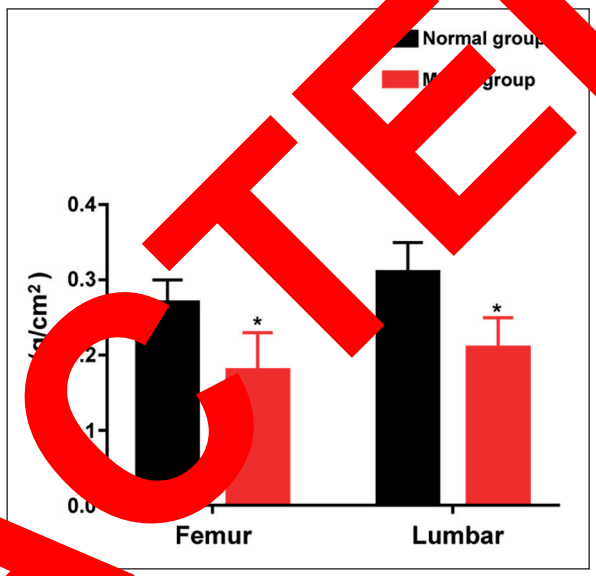
Name	Primer sequence
TAZ	Forward primer: 5'ATGTTGACCTCCGGACTTTGG3' Reverse primer: 5'GAGGAAGGGCTCGCTTTTGT 3'
GAPDH	Forward primer: 5'ACGGCAAGTTCAACGGCACAG 3' Reverse primer: 5'GAAGACGCCAGTAGACTCCACGA

heterogeneity of variance, and non-parametric test for those not meeting normal distribution and homogeneity of variance. The rank-sum test was utilized for the ranked data and the chi-square test for the enumeration data. $p<0.05$ was considered statistically significant.

Results

Detection of BMD

As shown in Figure 1, the BMD of the femur and lumbar was (0.27±0.03) and (0.31±0.04), respectively, in normal group, which was (0.18±0.05) and (0.21±0.04), respectively, in model group. There were significant differences betwee two groups ($p<0.05$), suggesting that the rat el of osteoporosis was established successfull this experiment.



BMD in each group. Note: $^{*}p<0.05$ *vs.* normal group.

ALP Staining for Osteoge is

It was displayed in Figur hat the LP-positive cells were in blue-viol dicati /osblasts. ALP-positive ells w in group but fewer i odel gr nd inhibitor group. Accordin e statistica lysis of the average optical density of ALP staining (Figure 3), the average optical density of ALP-positive cells was decreased markedly in model group and inhibitor group compared with that in normal

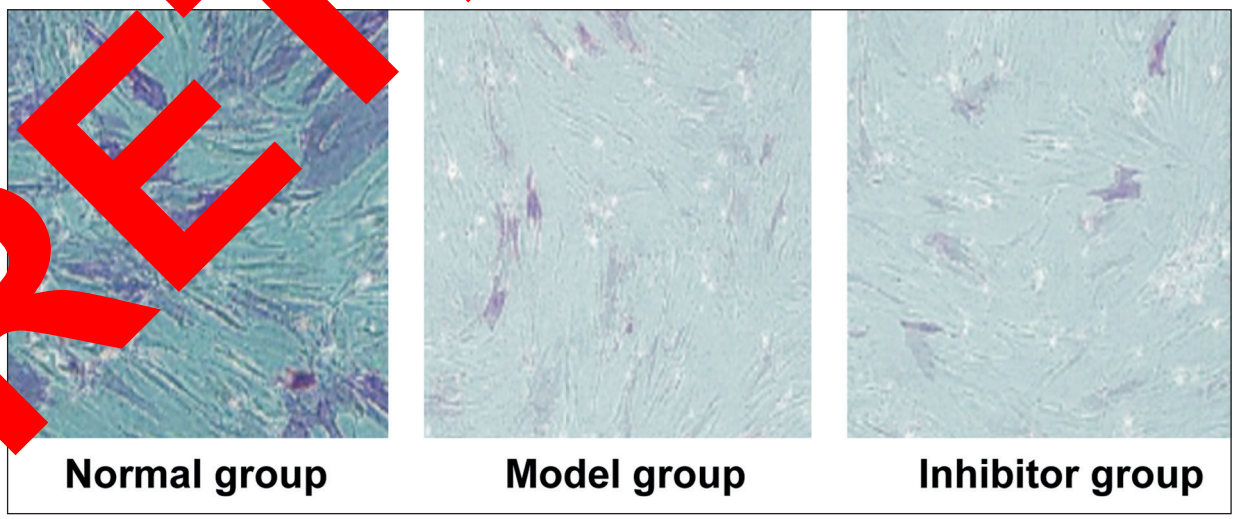


Figure 2. ALP staining in each group (×200).

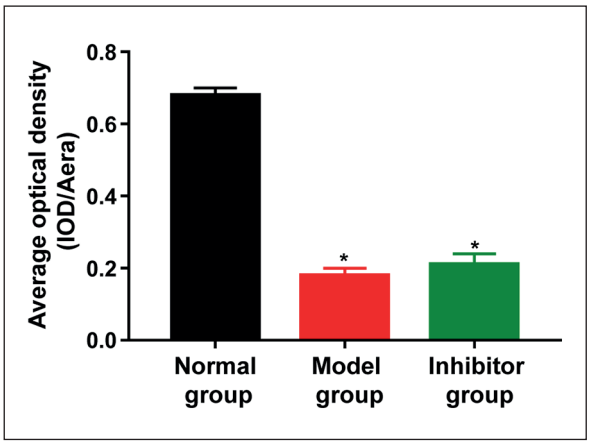


Figure 3. Average optical density of ALP-positive cells in each group. Note: $^{*}p<0.05$ *vs.* normal group.

group, with statistically significant differences ($p<0.05$). However, no significant difference in the average optical density of ALP-positive cells was discovered between model group and inhibitor group ($p>0.05$).

Expression of TAZ Detected Via Immunohistochemistry

The positive expression of TA in sepi There was a more positive ex TAZ i normal group and inhibitor up an ss posi-tive expression of TAZ in model group (Figure 4). The statistical results (Figure 5) manifested that the average optical density of cells with TAZ positive expression declined significantly in model group in comparison with that in normal g displaying a statistically significant di ence ($p<0.05$). The difference in the aver optical density of cells with TAZ positive exp n between normal group and inhibito roup not significant ($p>0.05$).

Relative Expressions Relate roteins Detected Via Western tt

As shown in F e 6, al gro ad higher expressio p-Akt an P-2 oteins than model gr inhibitor gro cording to the statis l re (Figure 7), he relative expressions of p-Akt BMP-2 proteins were reduc rkably in n group and inhibitor up compared with th e in normal group, ar he differences were statistically significant (05). There e no significant differences in relative e ssions of p-Akt and BMP-2 p bet model group and inhibitor group (The expression of TAZ mRNA was decreased in model group in comparison with that nor l group, displaying a statistically significant difference ($p<0.05$), while no significant difference was detected between normal group and inhibitor group ($p>0.05$) (Figure 8).

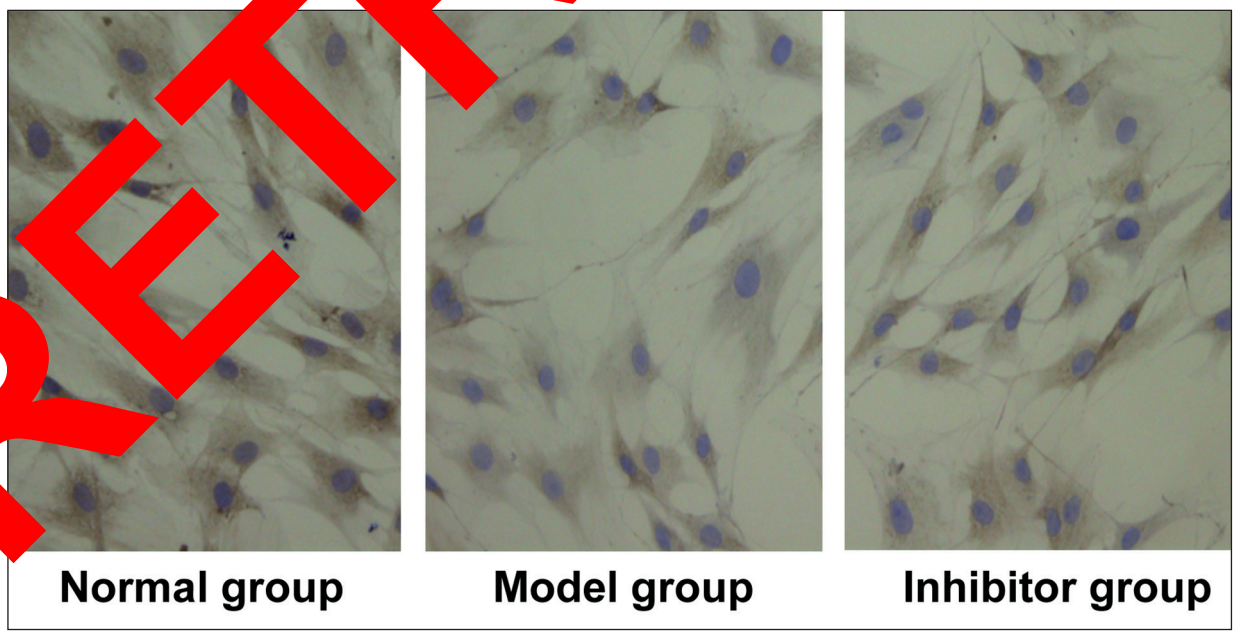


Figure 4. Positive expression of TAZ in each group (×200).

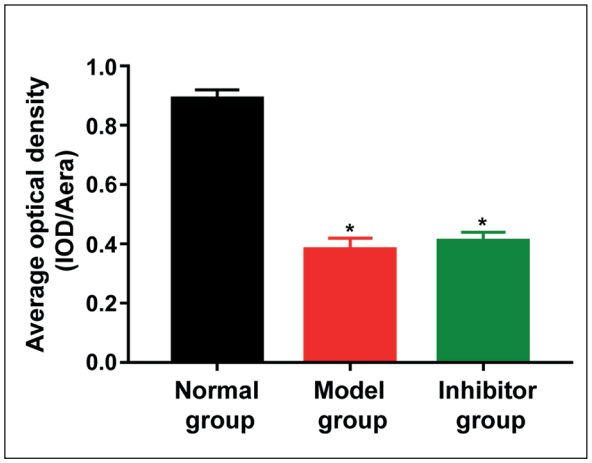


Figure 5. Average optical density of TAZ positive expression. Note: $^{*}p<0.05$ *vs.* normal group.

Cell Proliferation Detected Via CCK-8

Model group and inhibitor group had prominently higher cell proliferation rates than normal group, with statistically significant differences ($p<0.05$). Compared with that in model group, the cell proliferation rate was lowered in inhi group, and the difference was statistically s icant ($p<0.05$) (Figure 9).

Discussion

Osteoporosis is a seve isease ing the health and quality life incidence rate is g year ear with the progression of p tion aging i ociety. In particular, the rate of osteop sis is extremely high among t stmenopausal elderly women, complicatio duced thereby, such as pai d osteoporotic fra , seriously affect

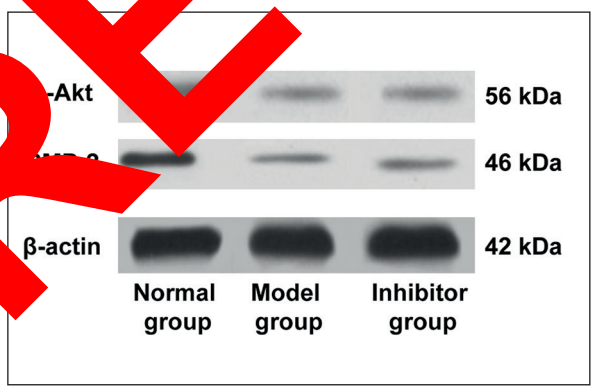


Figure 6. Protein expressions in each group.

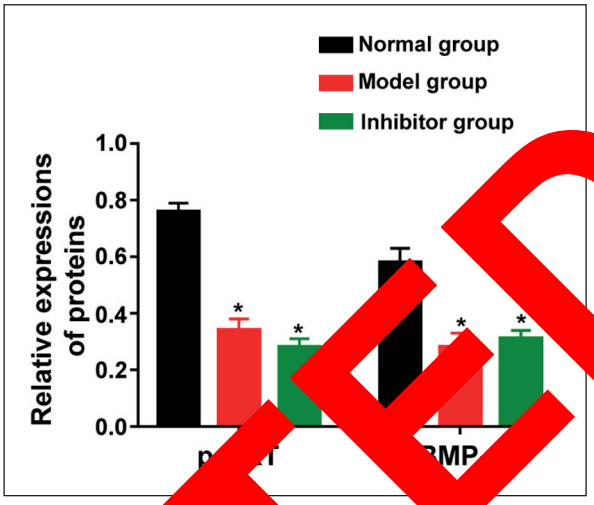


Figure 7. Re ve e sions of pro ns in each group. Note: $^{*}p<0.05$ v mal group.

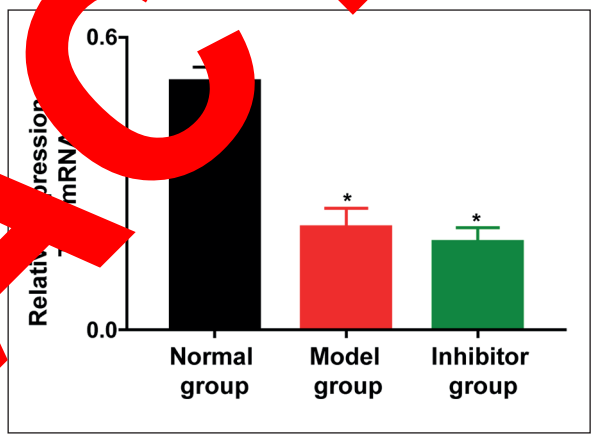


Figure 8. Relative expression of TAZ mRNA in each group. Note: $^{*}p<0.05$ *vs.* normal group.

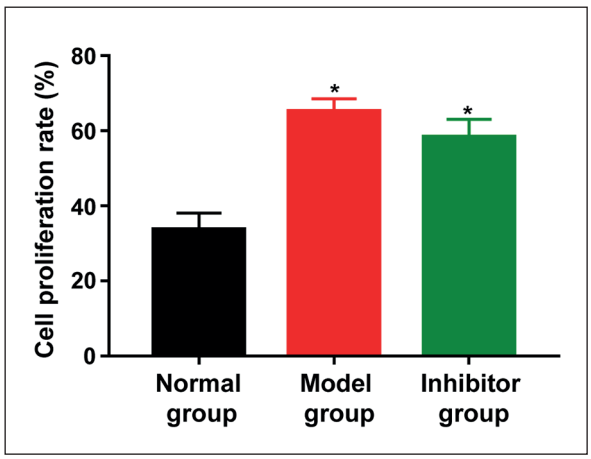


Figure 9. Cell proliferation rate in each group. Note: $^{*}p<0.05$ *vs.* normal group.

the patient's physical and mental health, as well as quality of life. Besides, substantial human, material, and financial resources have been put into the prevention and treatment of the disease by the whole society[7]. Therefore, it is of significant importance for the clinical treatment of osteoporosis to deeply study its pathogenesis. Currently, it is argued that the occurrence of osteoporosis is closely correlated with bone metabolism disorder caused by the dysfunctions and disproportions of osteoclasts and osteoblasts in the body. Reports[8,9] have revealed that the content of estrogen in postmenopausal women declined dramatically, which results in osteoclast hyperfunction and osteoblast inhibition in the body, thus enabling the osteoclast-induced bone destruction to significantly exceed the osteoblast-induced bone formation and leading to osteopenia. Meanwhile, the bone marrow mesenchymal stem cells are the major source of osteoblast and osteoclast differentiation in the body, while the proportion of massive bone marrow mesenchymal stem cells differentiating into the osteoblasts is decreased under the stimulation of reduced estrogen, further influencing the bone formation and leading to osteoper... this experiment, castration of male and fe... rats was adopted to remove their ovaries to c... an abrupt decrease in the estrogen. At 12 we... after modeling, the BMD of femu... lumbar ... the rats was detected and it ... that the BMD of the castrated rats ... ined p... inently, illustrating that the metho... prep... rat model of osteoporosis ... fea... was successfully es...ished in ... experiment.

As a vital cat... of stem ce... the body, characterized ... ation and di... ntiation, the bone ma... ow me... ymal stem cells can differenti... into cell typ... ith specific actions, such a... teoblasts and oste... ts[10]. It is currently ... ved th... many important cells signaling p... ays ... cipate in controlling the fate of bon... mesen... mal stem cells, in which they ca... ulate ... cells to maintain in the pro... rative ... o differentiate toward a certain ... e of cell... he PI3K/Akt signaling pathway, ... ial cell signal transduction pathway, has ... ed to play important regulatory roles in such physiological and pathological processes as cell proliferation, differentiation, apoptosis, and necrosis. Researches[11,12] have discovered that the PI3K/Akt cell signaling pathway can activate β-catenin and its phosphorylation. Also, it can promote the transcription of target genes and translation of proteins in downstream signaling pathways. In the meantime, the p-Akt produced by the phosphorylation of Akt in the PI3K/Akt signaling pathway is capable of promoting the phosphorylation of β-catenin and triggering cell proliferation[13,14]. In a word, the PI3K and ... in the cells can be activated by variou... nulations to recruit and activate P110 ... nd the cells, thereby exerting effects on and ... ating phosphatidylinositol-bisphosphat... n the ... ace of the cell membrane, formin... arge numb... f phosphatidylinositol-triphos... te[15]. A... a seco... messenger, phosphatidyli... ol-tri... phate can further activate the phosp... d... n of Ak... to generate p-Akt, th... playi... role in ... nal transduction, ex... g synergis... fec... *via* the downstream G... nd Wnt path... and regulating the ... urren... f cell differentiation[16,17]. Therefore, the PI3K/A... ignaling pathway is capab... ontrolling th... e of bone marrow me... nymal stem cells, w... ose activation can ac... erate the differentiation of bone marrow m... nchymal st... cells, and its repression can reg... e the prol... ation of those cells. According ... resul... this experiment, in the bone marrow ... ymal stem cells of osteoporosis ..., the PI3K/Akt signaling pathway was inhibexpression level of p-Akt protein was ...creased notably, and the cell proliferation rate was remarkably higher than that in normal bone marrow mesenchymal stem cells. As a result, it is essential to effectively regulate the PI3K/Akt signaling pathway to facilitate the differentiation of bone marrow mesenchymal stem cells into osteoblasts.

TAZ, a kind of transcriptional coactivator and a vital effector molecule, can affect multiple downstream cell signaling pathways, thereby exerting crucial regulatory effects in cell proliferation, differentiation, and other physiological processes[18,19]. In this research, the expressions of TAZ and TAZ mRNA, as well as the proliferation rate of bone marrow mesenchymal stem cells in osteoporosis rats, were markedly lower than those in normal bone marrow mesenchymal stem cells, indicating that the expression of TAZ is abnormal in the bone marrow mesenchymal stem cells of osteoporosis rats, thus leading to persistent cell proliferation and weakened osteogenic capacity. Taken together, these are all involved in the pathogenesis of osteoporosis. It was further discovered that after applying the inhibitor of the PI3K/Akt signaling pathway, the effect of TAZ on the bone marrow mesenchymal stem cells was attenuated prominently, the

osteogenic differentiation capacity of normal bone marrow mesenchymal stem cells was decreased, and their proliferation capacity was enhanced. Therefore, TAZ promotes the osteogenic differentiation of mesenchymal stem cells in the rat model of osteoporosis by repressing the PI3K/Akt signal.

Conclusions

We demonstrated that TAZ promotes the osteogenic differentiation of mesenchymal stem cells in the rat model of osteoporosis by repressing the PI3K/Akt signal.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Pietschmann P, Mechtcheriakova D, Meshcheryakova A, Foger-Samwald U, Ellinger I. Immunology of osteoporosis: a mini-review. Gerontology 2016; 62: 128-137.
2) Frank E. Treatment of low bone density or osteoporosis to prevent fractures in men and women. Ann Intern Med 2017; 167: 899.
3) An J, Yang H, Zhang Q, Liu C, Zhang Y, Chen B. Natural products for treatment of osteoporosis: the effects and mechanisms on promoting osteoblast-mediated bone formation. Life Sci 2016; 147: 46-58.
4) Yu JS, Cui W. Proliferation, differentiation and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. Development 2016; 143: 3050-3060.
5) Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S. Role of YAP/TAZ in mechanotransduction. Nature 2011; 474: 179-183.
6) Kong JH, Yang ES, McManus MT, Amsterdam A, Y[illegible]mukova R, Mueller E, Benjamin T, Spiegel[illegible], Sharp P, Hopkins N, Yaffe MB. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. Science 2005; 309: 1074-1078.
7) Adler RA, Hajj Fuleihan G, Bauer DC, Camacho PM, Clarke BL, Clines GA, Compston JE, Drake MT, Edwards BJ, Favus MJ, Greenspan SL, McKinney R Jr, Pignolo RJ, Sellmeyer DE. Managing osteoporosis in patients on long-term bisphosphonate treatment: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res 2016; 31: 16-35.
8) Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, O'Karma M, Wallace TC, Zemel BS. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int 2016; 27: 1281-1386.
9) Qiu Y, Yang W, Wang Q, Yan S, Li B, Zhai[illegible] teoporosis in postmenopausal women i[illegible]s decade: a bibliometric assessment of[illegible]ent research and future hotspots. Arch [illegible]oporos 2018; 13: 121.
10) Krampera M, Cosmi L, Angeli [illegible]asini A, Li[illegible]F, Andreini A, Santarlasci V, M[illegible]nghi B, Pizzolo Vinante F, Romagnani P, M[illegible] E, Ro[illegible]ani S, A[illegible] nunziato F. Role for in[illegible]on-ga[illegible] in the immunomodulatory activity [illegible] bone ma[illegible]ow mesenchymal st[illegible]cells. [illegible]Cells 20[illegible]24: 386-398.
11) Sami A, Ka[illegible]rgeting the [illegible]T/mTOR signalin[illegible]nw[illegible] glioblastoma[illegible]ovel therapeutic ag[illegible]nts and[illegible]ances in understanding. Tumour Biol 2013; 34[illegible]-2002.
12) [illegible]C, Chen N, Gu [illegible]n A, Cao L, Wang E, [illegible]ang L. PI3K/Akt/mTOR signaling pathway and targeted therapy for glioblastoma. Oncotarget 2016; 7: 3344[illegible]3450.
13) [illegible]errero-Zotan[illegible]A, Mayer IA, Arteaga CL. PI3K/AKT/mTOR: [illegible] in breast cancer progression, d[illegible], and treatment. Cancer Metastasis Rev 2016; 35: 515-524.
14) [illegible]ayad SE, Cohen PA. Inhibitors of the PI3K/Akt/mTOR pathway: new hope for breast cancer patients. Recent Pat Anticancer Drug Discov 2010; 5: 29-57.
15) Wang H, Zhang C, Xu L, Zang K, Ning Z, Jiang F, Chi H, Zhu X, Meng Z. Bufalin suppresses hepatocellular carcinoma invasion and metastasis by targeting HIF-1alpha via the PI3K/AKT/mTOR pathway. Oncotarget 2016; 7: 20193-20208.
16) Yang X, Song X, Wang X, Liu X, Peng Z. Down-regulation of TM7SF4 inhibits cell proliferation and metastasis of A549 cells through regulating the PI3K/AKT/mTOR signaling pathway. Mol Med Rep 2017; 16: 6122-6127.
17) Baek SH, Ko JH, Lee JH, Kim C, Lee H, Nam D, Lee J, Lee SG, Yang WM, Um JY, Sethi G, Ahn KS. Ginkgolic acid inhibits invasion and migration and TGF-β-induced EMT of lung cancer cells through PI3K/Akt/mTOR inactivation. J Cell Physiol 2017; 232: 346-354.
18) Lei QY, Zhang H, Zhao B, Zha ZY, Bai F, Pei XH, Zhao S, Xiong Y, Guan KL. TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway. Mol Cell Biol 2008; 28: 2426-2436.
19) Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, Inui M, Montagner M, Parenti AR, Poletti A, Daidone MG, Dupont S, Basso G, Bicciato S, Piccolo S. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell 2011; 147: 759-772.