European Review for Medical and Pharmacological Sciences 2019; 23: 5558-5566

Effects of lncRNA DANCR on proliferation and differentiation of osteoblasts by regulating the Wnt/β-catenin pathway

S.-Y. JIANG[1], Y.-X. MIAO[2], T. HIROKAZU[3], S.-Z. ZHU[4], J.-S. LU[1]

[1]Department of Traumatic Orthopedics, People's Hospital of Dongying City Shandong Province, Dongying, China
[2]Operating Room, People's Hospital of Dongying City Shandong Province, Dongying, China
[3]Department of Orthopedics, Fuji toranomon Orthopedic Hospital, Japan
[4]Laboratory Medicine, Qilu Hospital of Shandong University (Qingdao), Qingdao, China

Abstract. – OBJECTIVE: To measure the expression level of long non-coding ribonucleic acids (lncRNAs) differentiation antagonizing non-protein coding RNA (DANCR) in serum of patients with fracture and investigate its influences on the proliferation and differentiation of osteoblasts.

PATIENTS AND METHODS: Serum samples were collected from 44 fracture patients treated in our hospital and 24 healthy people receiving physical examination in our hospital. Then, reverse transcription-polymerase chain reaction (RT-PCR) technique was used to detect the expression of lncRNA DANCR in the serum of patients with fracture and healthy subjects. MC3T3-E1 mouse osteoblast cell line with stably-knocked out DANCR was further established using small interfering RNAs (siRNAs), and the effect of DANCR knockout on the proliferation of osteoblasts was determined using cell counting kit-8 (CCK-8). At the same time, 5-Ethynyl-2'-deoxyuridine (EdU) staining assay was performed to detect the percentage of EdU-positive cells in osteoblasts in control group and DANCR knockout group. In addition, the mRNA levels of differentiation-related genes including Runt-related transcription factor 2 (Runx2), Collagen1α1, osteocalcin (OC) and osterix (OSX) were detected *via* RT-PCR, and the protein level of Runx2 was measured through Western blotting. Moreover, osteoblasts were cultured with osteogenic medium for 14 d, and then alizarin red staining and alkaline phosphatase (ALP) staining assays were carried out to examine the differentiation of these osteoblasts. Lastly, Western blotting technique was employed to analyze the expression of the Wnt/β-catenin signaling pathway.

RESULTS: The expression of lncRNA DANCR was significantly increased in the serum of fracture patients ($p<0.05$). The results of *in-vitro* cell experiments showed that the intervention of DANCR with siRNA was able to clearly promote the proliferation and differentiation of MC3T3-E1 osteoblast cell line. According to the results of Western blotting, DANCR promoted the apoptosis and proliferation, which was mediated by the activated Wnt/β-catenin signaling pathway in osteoblasts.

CONCLUSIONS: LncRNA DANCR inhibition can facilitate the proliferation and differentiation of osteoblasts by activating the Wnt/β-catenin signaling pathway in osteoblasts. Therefore, DANCR is expected to be a new target promoting fracture healing.

Key Words:
LncRNA DANCR; Osteoblasts; Proliferation; Differentiation

Introduction

The bone is an organ undergoing sustainable reconstruction. During the reconstruction, osteoblasts and osteoclasts play important roles[1]. Many bone diseases like osteoporosis are caused by increased osteoclast activity or decreased number of osteoblasts[2]. Moreover, bone healing is a complex and dynamic process regulated by various cellular components and cytokines, during which osteoclasts are responsible for cartilage absorption and reconstruction by secreting acid and protease, while osteoblasts are mainly in charge of new bone formation[3]. In addition, increased production of osteoclasts can accelerate cartilage reabsorption and promote bone union formation during fracture healing, while inhibited differentiation of osteoblasts or osteoclasts suppresses bone healing[4, 5]. A study manifested that the production of both osteoblasts and osteoclasts is regulated by a variety of genes or proteins, so that designing drugs with these genes or proteins as targets will provide new ideas for accelerating fracture healing[6].

Corresponding Author: Shanyong Jiang, BM. e-mail: 13571147@qq.com

Long noncoding ribonucleic acids (lncRNAs) are long-stranded RNA molecules with over 200 nucleotides in transcription length[7]. They cannot encode corresponding proteins by themselves in cells, but can regulate the expressions of target genes at a (post-) transcriptional level or through epigenetic modification, ultimately affecting the development and progression of diseases[8, 9]. As a member of the lncRNA family, lncRNA differentiation antagonizing non-protein coding RNA (DANCR) plays a vital role in many diseases including tumors, cardiovascular diseases, endocrine diseases and bone diseases[10-12]. For instance, lncRNA DANCR is able to repress the differentiation of osteoblasts by promoting the degradation of Skp2-induced FOXO1 ubiquitination after total hip arthroplasty[13]. In addition, lncRNA DANCR suppresses odontoblast-like differentiation of human dental pulp cells by inhibiting the activation of the Wnt/β-catenin pathway[14]. However, there is no study on the role of lncRNA DANCR in bone healing at present.

In this study, the difference in the lncRNA DANCR expression in serum between healthy people and fracture patients was detected firstly, the influences of lncRNA DANCR knockout on the proliferation and differentiation of MC3T3-E1 osteoblastic cell line were then determined, and the molecular mechanism leading to such a difference was explored lastly.

Patients and Methods

Serum samples

A total of 44 fracture patients aged (47.87±5.39) years old and treated in our hospital from December 2017 to July 2018 were selected. In addition, 24 healthy people aged (44.21±7.89) years old and receiving medical examination were enrolled as control group. Then, venous blood (4 mL) was collected, added with sodium citrate for anticoagulation and stored in a refrigerator at -20°C for later use. This study was approved by the Ethics Committee of our hospital, and all subjects signed the informed consent.

Cell culture

MC3T3-E1 osteoblast cell line (purchased from Bioleaf, Shanghai, China) was cultured in complete α-MEM. At the same time, 10% fetal bovine serum and 1% penicillin streptomycin were added to the medium, followed by culture in a humidified atmosphere containing 95% air and 5% CO2 at 37.0°C. The medium should be replaced every 2-3 days.

Knockout of DANCR

MC3T3-E1 cells in the logarithmic growth phase were digested at once and inoculated in a 6-well plate cell culture dish. After 12 h (cell confluence reached 60-80%), the complete medium was discarded, and cells were washed with serum-free medium for 2-3 times and put in the incubator for starvation treatment, so as to achieve synchronous growth of cells. Next, DANCR small interfering RNAs (siRNAs) were dissolved into RNase-free water to prepare into solution with a final concentration of 20 μmol/L. Then, cells were divided into two groups, namely blank control group (Control group) and DANCR knockout group (DANCR siRNA group). The prepared transfection solution was added to each well in sequence and fully mixed for further culture for 6 h, followed by replacement with complete medium. The base sequences of DANCR siRNA are as follows: Forward: 5'- CGCAUCAGCUGCUACGCGG- 3', Reverse: 5'-CGUCGUAGCUGCUAGCAA-3'.

Detection of related gene expressions via reverse transcription-polymerase chain reaction (RT-PCR)

(1) Total RNA was extracted from each group using TRIzol method (Invitrogen, Carlsbad, CA, USA). Then, the concentration and purity of extracted RNA were detected using an ultraviolet spectrophotometer, and A_{260}/A_{280}=1.8-2.0 indicated that it was usable. (2) Messenger RNAs (mRNAs) were synthesized into complementary deoxyribonucleic acids (cDNAs) through reverse transcription and then stored in the refrigerator at -80°C. (3) RT-PCR system: 2.5 μL 10× buffer, 2 μL cDNA, 0.25 μL forward primer (20 μmol/L), 0.25 μL reverse primer (20 μmol/L), 0.5 μL DNTPs (10 mmol/L), 0.5 μL Taq enzyme (2×106 U/L) and 19 μL ddH_2O. The amplification systems of RT-PCR were same. The primer sequences of each gene are shown in Table I: Runt-related transcription factor 2 (Runx2), Collagen1α1, osteocalcin (OC) and osterix (OSX).

5-Ethynyl-2'-deoxyuridine (EdU) staining assay

After DANCR in MC3T3-E1 cells was knocked out using siRNAs for 24 h, MC3T3-E1 cells were stained using a Click-iT EdU staining kit (Invitrogen, Carlsbad, CA, USA) according to

Table I. Primer sequences of all indexes in RT-PCR

Target gene		Primer sequence
GAPDH	Forward	5'-GACATGCCGCCTGGAGAAAC- 3'
	Reverse	5'-AGCCCAGGATGCCCTTTAGT- 3'
DANCR	Forward	5 '-TGCTGCCTTTTCTGTTCCTT- 3'
	Reverse	5'-AAGGTGCTGGGTAGGGAAGT- 3'
Runx2	Forward	5'-GTCCAACCCGTAAGGT- 3'
	Reverse	5'-CGCTGCTGAGTCGATGCTAGCT- 3'
Collagen1α1	Forward	5'-ACGTAGCTAGCTAGTCGGTATG- 3'
	Reverse	5'-AAAACGTGGCTAGTCGATCG- 3'
OC	Forward	5'-ATCGTAGCTAGCTAGTCGAGCA- 3'
	Reverse	5'-CCCCCTGTGCTAGCTAGCTAGC- 3'
OSX	Forward	5'-GTGCTGATGTTAGCTAGCTAGCT- 3'
	Reverse	5'-AGCTAGTCGTAGCTAGCTGATCG- 3'

its instructions for specific operation procedures. After that, a fluorescence microscope was used to take pictures, with 3 fields randomly selected for each slide. Lastly, the number of EdU-positive cells was counted and quantified.

Cell counting kit-8 (CCK-8) proliferation assay

Cells in the logarithmic growth phase in each group were inoculated into a 96-well plate and cultured in a thermostatic incubator at 37°C and 5% CO_2 for 0, 24, 48 and 72 h, followed by discard of the medium. Next, color-developing solution was prepared using 1640 medium and CCK-8 (Dojindo, Kumamoto, Japan) at a ratio of 10:1 (in a dark place) and then added to the 96-well plate with 110 μL for each well. Thereafter, the plate was incubated in the thermostatic incubator for 2 h. Lastly, the ultraviolet spectrophotometer was utilized to read the optical density at 450 nm (OD_{450}) in each group.

Western blotting assay

(1) The medium was discarded firstly, and cells were then washed with phosphate-buffered saline (PBS) for three times. (2) Lysis solution (1,000 μL) was added to each dish, followed by sufficient shaking for 20 min. (3) Cells at the bottom of the dish were scraped off with a brush and put into a prepared Eppendorf (Ep) tube. (4) Collected cells were subjected to lysis using an ultrasonic cracker for about 15 s. (5) After still standing for 15 min, cells were centrifuged for 0.5 h (at 12,000r/min). (6) The supernatant was collected and dispensed into the Ep tube, protein concentration was measured by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA) and ultraviolet spectrophotometry, and the concentration of protein of all samples was adjusted to the same. (7) Protein was dispensed and placed in the refrigerator at -80°C. Total protein was extracted from osteoblasts, subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA) and incubated with primary antibody at 4°C overnight and then goat anti-rabbit secondary antibody at the dark for 1 h. Thereafter, protein bands were scanned and quantified using an Odyssey scanner. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to correct protein levels to be measured.

Alizarin red staining assay

Cells in each group were washed twice with pre-cooled PBS, fixed with 4% paraformaldehyde for 10 min and incubated with 30 mM alizarin red S (pH=4.2, Sigma, St. Louis, MO, USA) at room temperature for 10 min, followed by photographing using a camera.

Alkaline phosphatase (ALP) staining assay and activity determination

Cells in all groups were washed with PBS for two times, fixed with 4% paraformaldehyde for 10 min, rinsed with deionized water, and stained for 1 h using a BCIP/NBT ALP chromogenic kit (Beyotime, Shanghai, China) according to its instructions. Then, the camera was utilized to take pictures. Activity determination: Osteoblasts were cultured with medium for 14 d, and then washed with PBS, followed by lysis. Cell lysates were centrifuged, and the supernatant was retained. ALP activity in the supernatant was detected using a kit (Sigma-Aldrich, St. Louis, MO, USA) in accordance with its instructions,

followed by quantification using a BCA protein determination kit (Pierce, Rockford, IL, USA).

Statistical analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used to analyze all data. Measurement data were represented as mean ± standard deviation, and *t*-test was employed for comparisons of data between two groups. $p<0.05$ suggested that the difference was statistically significant.

Results

Expression of lncRNA DANCR in the plasma of healthy people and fracture patients

The level of lncRNA DANCR in the serum of subjects in both groups was firstly measured *via* RT-PCR in this study, and it was found that the expression level of lncRNA DANCR in the serum of fracture patients was significantly (about 7.34 times) higher than that of healthy people ($p<0.05$) (Figure 1).

Construction of lncRNA DANCR-knocked out cell line

To further investigate the role of DANCR in fracture healing, DANCR in MC3T3-E1 cells was knocked out using siRNAs in this study, and then RT-PCR was performed to detect the efficiency of DANCR knockout in MC3T3-E1 cells. The results revealed that compared with that in Control group, the expression level of DANCR was reduced by 88.73% in DANCR siRNA group ($p<0.05$) (Figure 2).

Results of EdU staining of two groups of cells

To further determine the impact of DANCR knockout on the proliferation of cells in the two groups, EdU staining assay was carried out to evaluate the proliferative capacity of cells in each group. According to Figure 3, the number of EdU-positive cells in DANCR siRNA group was about 7.44 times higher than that in Control group ($p<0.05$), suggesting that silencing lncRNA DANCR can effectively inhibit the proliferation of osteoblasts.

Effect of lncRNA DANCR knockout on osteoblasts detected via CCK-8 proliferation assay

CCK-8 proliferation assay was conducted in this study to detect the OD_{480} value of two group

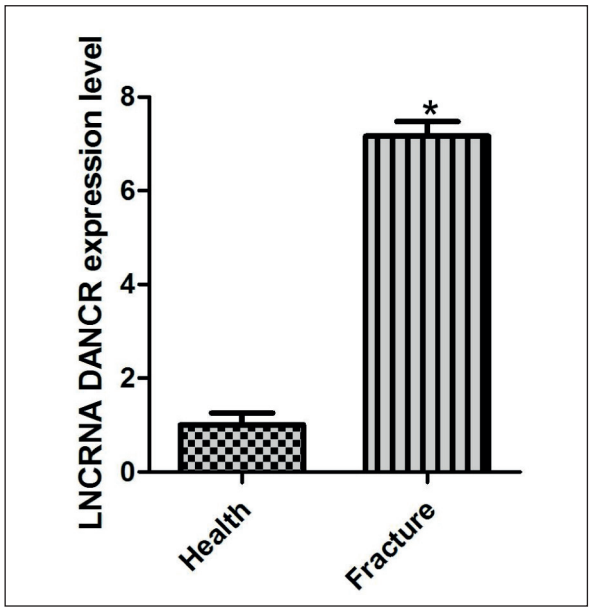

Figure 1. Expression of lncRNA DANCR in plasma of fracture patients. Health: Healthy controls, Fracture: Fracture patients. *: There is a statistical difference compared with Health group ($p<0.05$).

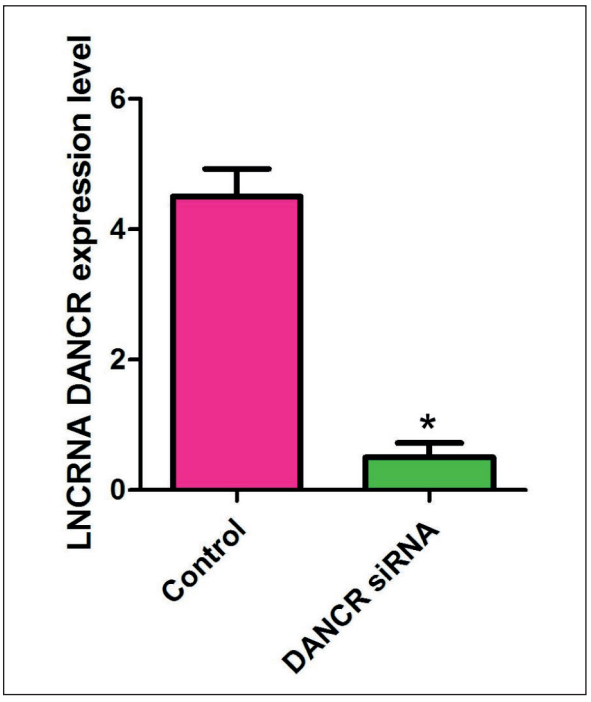

Figure 2. Knockout of lncRNA DANCR detected. Control: Blank control group, DANCR siRNA: DANCR knockout group. *: There is a statistical difference compared with Control group ($p<0.05$).

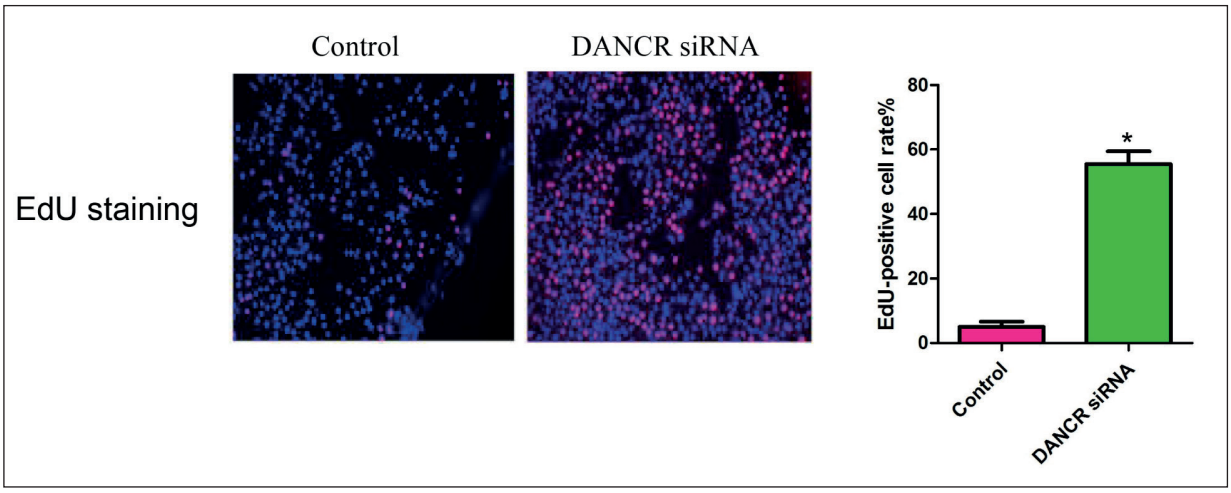

Figure 3. Results of EdU staining of two groups of cells. Control: Blank control group, DANCR siRNA: DANCR knockout group. *: There is a statistical difference compared with Control group (p<0.05).

of cells at 0, 24, 48 and 72 h to reflect the proliferative capacity of cells. It was discovered that the proliferative capacity of osteoblasts in DANCR siRNA group was overtly higher than that in Control group at 0, 24, 48 and 72 h (p<0.05) (Figure 4).

Influence of DANCR knockout on differentiation of osteoblasts detected through alizarin red and ALP staining assays

To evaluate the role of DANCR gene in the differentiation of osteoblasts, alizarin red and ALP staining assays were carried out in this study to detect cell differentiation ability in each group, and the results manifested that DANR siRNA group had clearly increased cell differentiation ability (p<0.05) and ALP activity (p<0.05) in comparison with Control group (Figure 5).

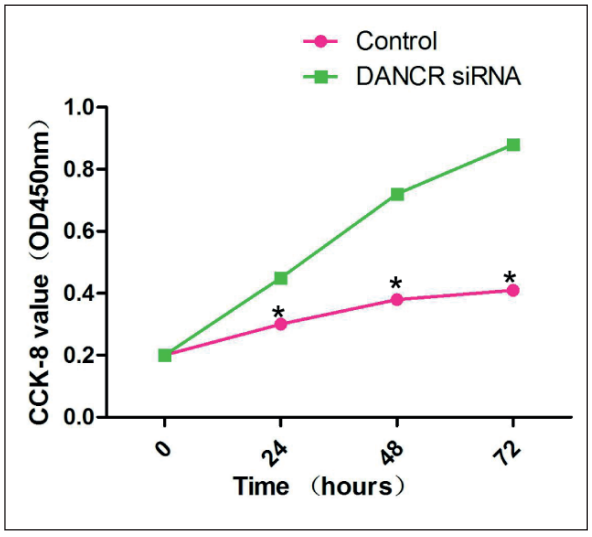

Figure 4. Proliferative capacity of cells detected via CCK-8. Control: Blank control group, DANCR siRNA: DANCR knockout group. *: There is a statistical difference compared with Control group (p<0.05).

Impact of DANCR knockout on differentiation of osteoblasts

Furthermore, the mRNA levels of osteoblast differentiation-related genes were measured in this study, and it was found that the mRNA levels of differentiation-related genes (Runx2, Collagen1α1, OC and OSX) in DANCR siRNA group were higher than those in Control group (p<0.05), indicating that DANCR siRNA group has evidently enhanced cell differentiation ability. At the same time, the protein level of Runx2 was measured *via* Western blotting in this study, and the results showed that DANCR siRNA also inhibited the expression of Runx2 at the protein level (Figure 6).

Role of DANCR knockout in the Wnt/β-catenin signaling pathway of osteoblasts

Considering that the Wnt/β-catenin signal pathway plays an important role in the proliferation and differentiation of osteoblasts, the protein expression levels of Wnt and β-catenin signaling pathways were measured through Western blotting technique in this study. The results revealed that the Wnt/β-catenin signaling pathway of osteoblasts was obviously activated after DANCR knockout (p<0.05) (Figure 7).

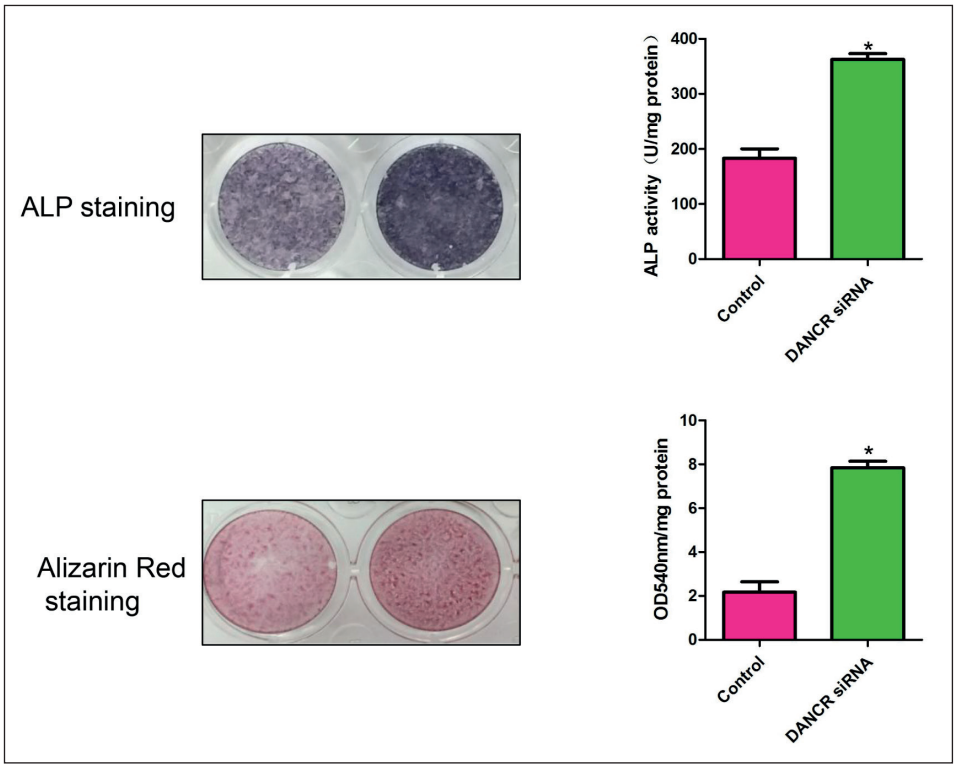

Figure 5. Influence of DANCR knockout on differentiation of osteoblasts detected through alizarin red and ALP staining assays. Control: Blank control group, DANCR siRNA: DANCR knockout group, *: There is a statistical difference compared with Control group (p<0.05).

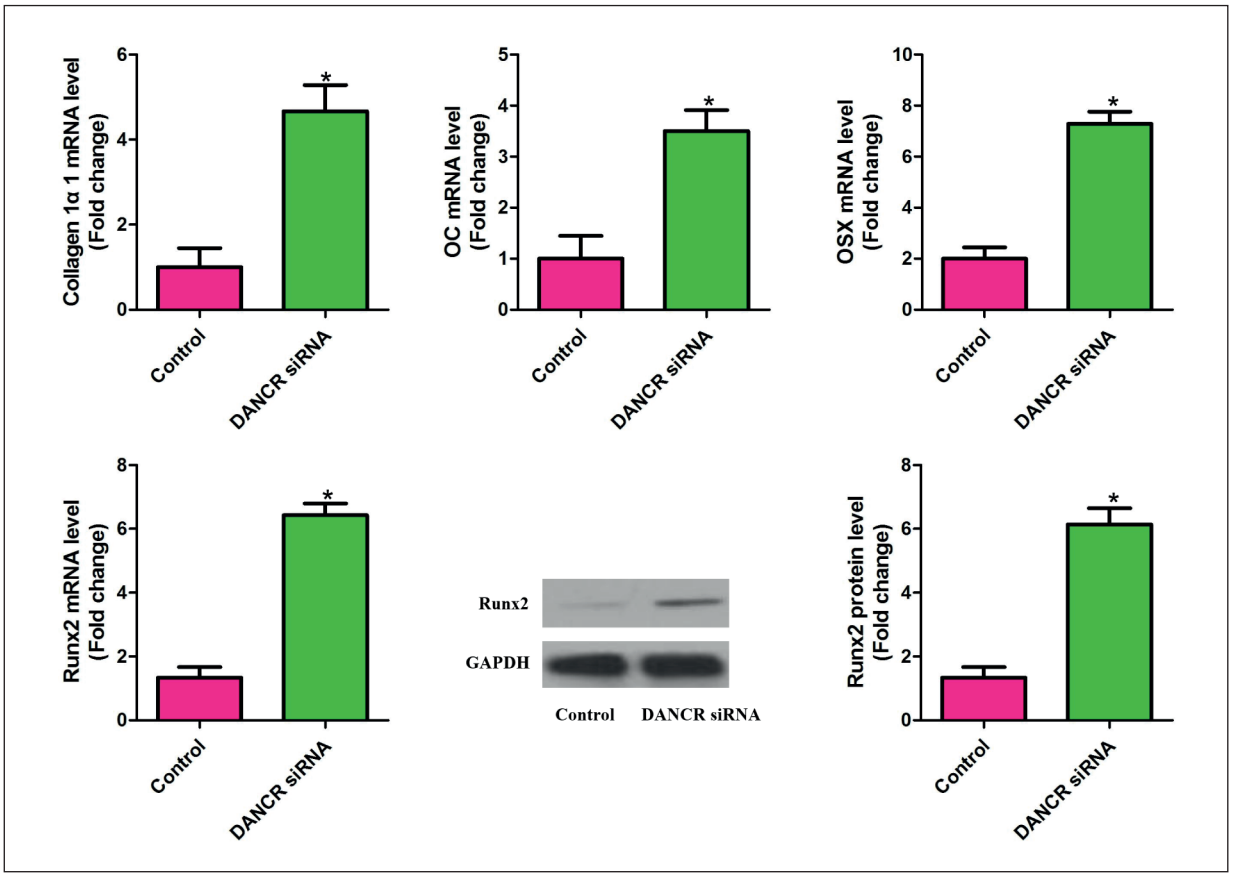

Figure 6. Impact of DANCR knockout on differentiation of osteoblasts. Control: Blank control group, DANCR siRNA: DANCR knockout group, *: There is a statistical difference compared with Control group (p<0.05).

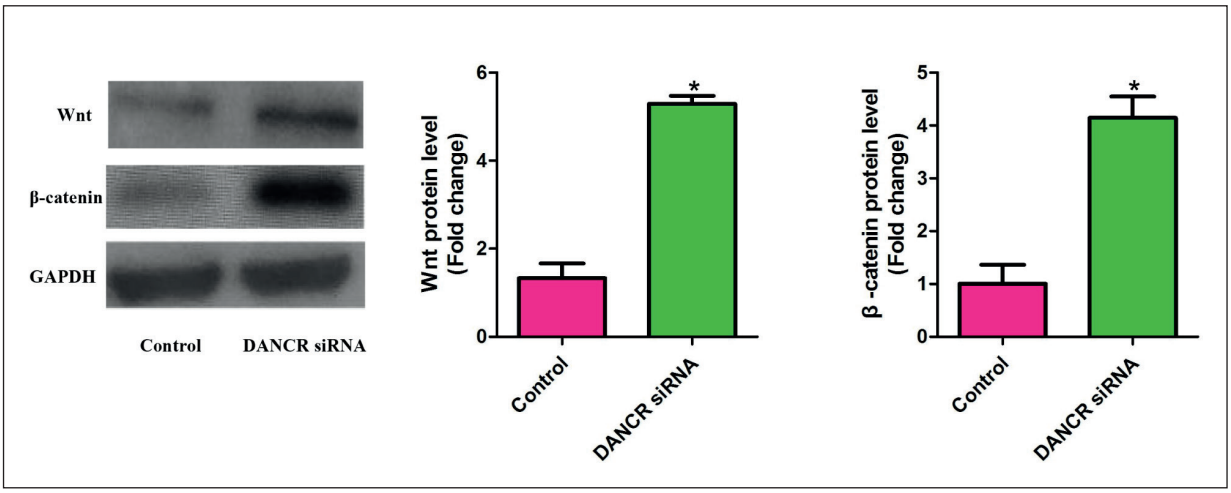

Figure 7. Role of DANCR knockout in the Wnt/β-catenin signaling pathway of osteoblasts. Control: Blank control group, DANCR siRNA: DANCR knockout group. *: There is a statistical difference compared with Control group ($p<0.05$).

Discussion

The bone is an organ being constantly reconstructed. Osteoblasts and osteoclasts play important roles in bone homeostasis and fracture healing[15]. Fracture healing is regulated by multiple cells and cytokines, and disturbance of osteoblasts/osteoclasts or microenvironment is sure to lead to abnormal fracture healing[16]. Endochondral ossification is an important physiological part of fracture healing and characterized by the replacement of cartilage callus with bone[17]. During the reconstruction of cartilage callus, inhibition due to osteoclasts can lead to disruption of chondrolysis, and suppressed proliferation and differentiation of osteoblasts is also able to cause slow formation of new bones, thus leading to enlarged bone lacuna of fracture, increased cartilage abnormalities and eventually delayed fracture healing[18]. A study showed that osteoblasts produce a variety of extracellular matrixes (including OCN, ALP, OPN and Collagen 1) that are the basis for maintaining bone homeostasis[19]. Moreover, the interruption of bone matrix deposition is sure to result in a series of bone diseases such as osteoporosis or osteopsathyrosis[20]. Therefore, it is essential to have an in-depth understanding of the molecular regulatory network of osteoblast proliferation and differentiation for the treatment of fractures.

Extensive genetic studies and genome-wide analyses have proved that the typical Wnt/β-catenin pathway plays a vital role in regulating bone homeostasis[21]. In addition, the Wnt/β-catenin signaling pathway is capable of regulating the differentiation of mesenchymal stem cells into osteoblasts[22]. Wnt protein outside the osteoblast binds to the Frizzled or LRP5/6 receptor on the envelope of the osteoblast to form a co-dimer through the interaction of various envelopes and plasmosin, promoting the accumulation of β-catenin in the cytoplasm and the entry into the nucleus to form a co-complex with the transcription factors TCF and LEF, thus transcribing and activating a series of genes promoting proliferation and differentiation (such as c-myc and cyclin D1)[23]. Activation of the Wnt/β-catenin signaling pathway is regulated by a series of genes or proteins including GSK-3β, intracellular dishevelled protein (Dsh) and the skelemin Axin. Therefore, the proliferation and differentiation of osteoblasts are effectively accelerated by up-regulating the Wnt/β-catenin signal of osteoblasts in numerous existing studies[24]. For instance, estrogen receptor-α is able to modulate the Wnt/β-catenin signaling pathway by binding to the coactivator PGC-1α, thereby promoting the differentiation of osteoblasts[25]. In addition, it has been confirmed that the traditional Chinese medicine geraniin can facilitate the proliferation and differentiation of osteoblasts by activating the Wnt/β-catenin pathway[26]. It has also been proved in models of various diseases that lncRNA DANCR is able to regulate the Wnt/β-catenin signaling pathway. For example, lncRNA DANCR significantly inhibits the upregulation of Wnt and β-catenin proteins and thus represses the differentiation of dental pulp cells into odontoblast-like cells[13]. Conversely, as to glioma, the overexpression of LncRNA DANCR promotes the proliferation and migration of glioma cells by activating the Wnt/β-catenin signaling pathway[27]. The results of this study are consistent with the

former findings. In this study, it was revealed for the first time that the serum expression level of lncRNA DANCR in fracture patients was clearly higher than that in healthy subjects. Furthermore, lncRNA DANCR knockout cell line was constructed in this study, and it was found that the levels of proliferation and differentiation of osteoblasts were significantly enhanced after silencing lncRNA DANCR, which were mainly reflected as increased percentage of EdU-positive cells and number of CCK-8-positive cells. At the same time, the mRNA levels of relevant markers (OSX, Runx2, Collagen1α1 and OC) promoting differentiation were overtly elevated. Finally, analyses on the expression levels of related proteins in the canonical Wnt/β-catenin signaling pathway showed that the Wnt/β-catenin pathway was evidently activated after lncRNA DANCR knockdown. Therefore, it is speculated that lncRNA DANCR affects the proliferation and differentiation of osteoblasts by up-regulating the Wnt/ The β-catenin signaling pathway. Even so, it is believed that this study still has some limitations: 1) Only one cell line (no primary cell or other cell line) was used, 2) Direct target genes of DANCR were not found, 3) No animal experiments were carried out for verification, 4) Only the canonical Wnt/β-catenin signaling pathway was tested. Non-canonical pathways also play important roles in the proliferation and differentiation of osteoblasts. Hence, whether DANCR affects the non-canonical pathways should be further verified.

Conclusions

The findings of this study substantiate that lncRNA DANCR plays an important regulatory role in the differentiation and proliferation of osteoblasts, which may be mediated by the Wnt/β-catenin signaling pathway.

Conflict of Interests

The authors declared no conflict of interest.

References

1) Amin NH, Hutchinson HL, Sanzone AG. Management of acute hip fracture. N Engl J Med 2018; 378: 972.
2) Kokabu S, Rosen V. BMP3 expression by osteoblast lineage cells is regulated by canonical Wnt signaling. FEBS Open Bio 2018; 8: 168-176.
3) Yu-Lee LY, Yu G, Lee YC, Lin SC, Pan J, Pan T, Yu KJ, Liu B, Creighton CJ, Rodriguez-Canales J, Villalobos PA, Wistuba II, de Nadal E, Posas F, Gallick GE, Lin SH. Osteoblast-secreted factors mediate dormancy of metastatic prostate cancer in the bone via activation of the TGFbetaRIII-p38MAPK-pS249/T252RB pathway. Cancer Res 2018; 78: 2911-2924.
4) Sanchez C, Mazzucchelli G, Lambert C, Comblain F, DePauw E, Henrotin Y. Comparison of secretome from osteoblasts derived from sclerotic versus non-sclerotic subchondral bone in OA: A pilot study. Plos One 2018; 13: e194591.
5) Kikuta J, Ishii M. Bone imaging: osteoclast and osteoblast dynamics. Methods Mol Biol 2018; 1763: 1-9.
6) Li W, Deng Y, Feng B, Mak KK. Mst1/2 kinases modulate glucose uptake for osteoblast differentiation and bone formation. J Bone Miner Res 2018; 33: 1183-1195.
7) Zhan M, He K, Xiao J, Liu F, Wang H, Xia Z, Duan X, Huang R, Li Y, He X, Yin H, Xiang G, Lu L. LncRNA HOXA11-AS promotes hepatocellular carcinoma progression by repressing miR-214-3p. J Cell Mol Med 2018:
8) Zhang CG, Yin DD, Sun SY, Han L. The use of lncRNA analysis for stratification management of prognostic risk in patients with NSCLC. Eur Rev Med Pharmacol Sci 2017; 21: 115-119.
9) Ding L, Ren J, Zhang D, Li Y, Huang X, Hu Q, Wang H, Song Y, Ni Y, Hou Y. A novel stromal lncRNA signature reprograms fibroblasts to promote the growth of oral squamous cell carcinoma via LncRNA-CAF/interleukin-33. Carcinogenesis 2018; 39: 397-406.
10) Liu Y, Zhang M, Liang L, Li J, Chen YX. Over-expression of lncRNA DANCR is associated with advanced tumor progression and poor prognosis in patients with colorectal cancer. Int J Clin Exp Pathol 2015; 8: 11480-11484.
11) Mao Z, Li H, Du B, Cui K, Xing Y, Zhao X, Zai S. LncRNA DANCR promotes migration and invasion through suppression of lncRNA-LET in gastric cancer cells. Biosci Rep 2017; 37:
12) Zhang L, Yang C, Chen S, Wang G, Shi B, Tao X, Zhou L, Zhao J. Long noncoding RNA DANCR Is a positive regulator of proliferation and chondrogenic differentiation in human synovium-derived stem cells. DNA Cell Biol 2017; 36: 136-142.
13) Tang Z, Gong Z, Sun X. LncRNA DANCR involved osteolysis after total hip arthroplasty by regulating FOXO1 expression to inhibit osteoblast differentiation. J Biomed Sci 2018; 25: 4.
14) Chen L, Song Z, Huang S, Wang R, Qin W, Guo J, Lin Z. lncRNA DANCR suppresses odontoblast-like differentiation of human dental pulp cells by inhibiting wnt/beta-catenin pathway. Cell Tissue Res 2016; 364: 309-318.
15) Bandeira F, Cusano NE, Silva BC, Cassibba S, Almeida CB, Machado VC, Bilezikian JP. Bone disease in primary hyperparathyroidism. Arq Bras Endocrinol Metabol 2014; 58: 553-561.
16) Ghiasi MS, Chen J, Vaziri A, Rodriguez EK, Nazarian A. Bone fracture healing in mechanobiological modeling: A review of principles and methods. Bone Rep 2017; 6: 87-100.

17) Wang Y, Malcolm DW, Benoit D. Controlled and sustained delivery of siRNA/NPs from hydrogels expedites bone fracture healing. Biomaterials 2017; 139: 127-138.
18) Kostenuik P, Mirza FM. Fracture healing physiology and the quest for therapies for delayed healing and nonunion. J Orthop Res 2017; 35: 213-223.
19) Hara Y, Ghazizadeh M, Shimizu H, Matsumoto H, Saito N, Yagi T, Mashiko K, Mashiko K, Kawai M, Yokota H. Delayed expression of circulating TGF-beta1 and BMP-2 Levels in human nonunion long bone fracture healing. J Nippon Med Sch 2017; 84: 12-18.
20) Cheung WH, Miclau T, Chow SK, Yang FF, Alt V. Fracture healing in osteoporotic bone. Injury 2016; 47 Suppl 2: S21-S26.
21) Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, Kharode Y, Sauter L, Babij P, Brown EL, Hill AA, Akhter MP, Johnson ML, Recker RR, Komm BS, Bex FJ. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. J Biol Chem 2006; 281: 31720-31728.
22) Li G, Su Q, Liu H, Wang D, Zhang W, Lu Z, Chen Y, Huang X, Li W, Zhang C, He Y, Fu L, Bi J. Frizzled7 promotes epithelial-to-mesenchymal transition and stemness via activating canonical wnt/beta-catenin pathway in gastric cancer. Int J Biol Sci 2018; 14: 280-293.
23) Li L, Peng X, Qin Y, Wang R, Tang J, Cui X, Wang T, Liu W, Pan H, Li B. Acceleration of bone regeneration by activating Wnt/beta-catenin signalling pathway via lithium released from lithium chloride/calcium phosphate cement in osteoporosis. Sci Rep 2017; 7: 45204.
24) Yao Q, Yu C, Zhang X, Zhang K, Guo J, Song L. Wnt/beta-catenin signaling in osteoblasts regulates global energy metabolism. Bone 2017; 97: 175-183.
25) Auld KL, Berasi SP, Liu Y, Cain M, Zhang Y, Huard C, Fukayama S, Zhang J, Choe S, Zhong W, Bhat BM, Bhat RA, Brown EL, Martinez RV. Estrogen-related receptor alpha regulates osteoblast differentiation via Wnt/beta-catenin signaling. J Mol Endocrinol 2012; 48: 177-191.
26) Li K, Zhang X, He B, Yang R, Zhang Y, Shen Z, Chen P, Du W. Geraniin promotes osteoblast proliferation and differentiation via the activation of Wnt/beta-catenin pathway. Biomed Pharmacother 2018; 99: 319-324.
27) Li J, Zhou L. Overexpression of lncRNA DANCR positively affects progression of glioma via activating Wnt/beta-catenin signaling. Biomed Pharmacother 2018; 102: 602-607.