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LncRNA KCNQ1OT1 delayed fracture healing through the Wnt/β-catenin pathway

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Abstract. – OBJECTIVE: The aim of this study was to explore the effect of long non-coding ribonucleic acid (lncRNA) KCNQ1 overlapping transcript 1 (KCNQ1OT1) on fracture healing and its possible mechanism.

PATIENTS AND METHODS: Abnormal lncRNAs were compared between patients with delayed fracture healing and those with normal fracture healing using gene expression profiling method. LncRNA expression in patients was verified by quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). Subsequently, the model of tibial fracture was successfully established in rabbits. The effect of lncRNA KCNQ1OT1 expression on tibial fracture healing in rabbits was explored. Meanwhile, the effects of lncRNA KCNQ1OT1 on cell proliferation and apoptosis were investigated by knockdown and overexpression experiments with HC-a as a cell model. Furthermore, Western blotting was used to explore the expressions of proteins in signaling pathway affected by lncRNA KCNQ1OT1.

RESULTS: Gene expression profiling and qRT-PCR revealed that lncRNA KCNQ1OT1 was significantly down-regulated in bone tissues of patients with delayed fracture healing. Compared with the control group, knocking down lncRNA KCNQ1OT1 remarkably reduced the serum levels of alkaline phosphatase (ALP) and osteoprotegerin (OPG) in rabbits, and markedly decreased bone trabecular growth index ($p<0.05$). In HC-a cells, overexpression of lncRNA KCNQ1OT1 activated the Wnt/β-catenin signaling pathway, which could be suppressed by knocking down lncRNA KCNQ1OT1. Cell Counting Kit-8 (CCK-8) assay and 5(6)-carboxyfluorescein diacetate, succinimidyl ester (CFSE) results manifested that lncRNA KCNQ1OT1 remarkably promoted the proliferation and inhibited apoptosis of HC-a cells by activating the Wnt/β-catenin signaling pathway.

CONCLUSIONS: LncRNA KCNQ1OT1 plays a vital role in delayed fracture healing. Moreover, it can induce cell proliferation and inhibit cell apoptosis by activating the Wnt/β-catenin signaling pathway. Therefore, KCNQ1OT1 may be used as a biomarker to predict the occurrence of delayed fracture healing.

Key Words:
LncRNA KCNQ1OT1, Wnt/β-catenin, Fracture healing.

Introduction

Serious fracture related to traffic accidents is a major health problem worldwide. Long-term treatment of fractures has a significant socio-economic impact[1]. Healing of some fractures are difficult and may take several months. Long-term treatment of fracture on working days is a major loss, which has economic impacts on patients and society. Currently, multiple risks of non-healing and disability have been found related to malunion, joint stiffness, muscular atrophy or reflex sympathetic dystrophy[2]. The purpose of clinical fracture treatment is to heal the bone in the shortest possible time, and to restore patients' motor ability to the best condition with fewer complications. However, the overall incidence rate of delayed healing or non-healing remains lower than 10%[3]. Currently, there is still a lack of biomarkers for effectively predicting delayed fracture healing and drug targets for therapeutic interventions. Therefore, searching for sensitive and reliable biomarkers that can be used to predict the occurrence of delayed fracture healing is necessary. This can eventually intervene in the early treatment of patients and help the recovery of fractures in time.

As endogenous cellular ribonucleic acid (RNA), long non-coding RNA (lncRNA) is a messenger RNA (mRNA)-like transcript with a length ranging from 200 nt to 100 kb[4]. Due to the lack of a significant open reading framework, lncRNA cannot be used as templates for protein synthesis. However, in current researches, several lncRNAs have been found to exert crucial roles in the pathophysiological process of various orthopedic diseases. For instance, lncRNA MEG3 inhibits osteogenic differentiation of bone

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marrow mesenchymal stem cells (MSCs) in postmenopausal osteoporosis by targeting microRNA (miR)-133a-3p. Meanwhile, it also plays a vital role in early and advanced osteoporosis cases[5]. Therefore, lncRNA HOXA11-AS can inhibit cell proliferation and promote osteoblast apoptosis by suppressing miR-124-3p, thereby participating in fracture healing[6]. At present, several reports have indicated that lncRNA KCNQ1 overlapping transcript 1 (KCNQ1OT1) promotes osteoblast differentiation by activating the Wnt/β-catenin signaling pathway. This helps to reduce osteolysis, thereby participating in the pathophysiological process of osteoporosis diseases[7]. However, no literature has elucidated the exact role of lncRNA KCNQ1OT1 in fracture healing. The aim of this study was to explore the role of lncRNA KCNQ1OT1 in fracture healing and the possible underlying mechanism.

Patients and Methods

Collection of Clinical Samples

20 tissues of delayed tibial fracture healing and 20 tissues of normal tibial fracture healing were collected during operation. 3 tissues obtained from patients with delayed tibial fracture healing and 3 tissues from those with normal tibial fracture healing were used for lncRNA microarray analysis. Immediately after collection, tissue samples were immersed in liquid nitrogen and stored in a refrigerator at -80°C for use. This study was approved by the Ethics Committee of The First People's Hospital of Qujing City. Signed informed consents were obtained from all participants before the study.

Gene Microarray Research

In this work, Affymetrix glue grant human transcriptome array (GG-H microarray) was used to analyze the expression levels of lncRNAs and mRNAs in tissue samples of patients by Gminix Information Technology Co., Ltd. (Shanghai, China)[8]. Gene set enrichment analysis (GSEA) software[9] (Broad Institute, http://www.broadinstitute.org/gsea/index.jsp) was applied to perform GSEA of differentially expressed genes. GSE entries used were derived from Molecular Signature Database v4.0 (Broad Institute, http://www.broadinstitute.org/gsea/index.jsp). Differentially expressed genes in gene microarrays were screened with $p<0.05$ and $|logFC|>2.0$ as screening criteria using R 3.5.2 software. Finally, differential expression heat map was drawn using LDheatmap Software Package (Burnaby, Canada) in R Language[10].

Total RNA Extraction and Detection of LncRNA Expression via Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA in tissues of patients in the two groups was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration of extracted RNA was measured by a Thermo 2000D ultraviolet spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The absorbance at 260 nm (A_{260}) was determined, and the ratio of A_{260}/A_{280} was used as an indicator of RNA purity. RNA samples with A_{260}/A_{280}=1.8 could be used for subsequent experiments. In addition, RNA quality and 28S and 18S bands were evaluated by electrophoresis on 1% agarose gel stained with ethidium bromide (EtBr). RNA samples with 28S bands twice as large as 18S bands were used in quantitative Reverse Transcription-Polymerase Chain Reaction qRT-PCR experiments.

The expression of lncRNA KCNQ1OT1 in tissue samples of delayed tibial fracture healing and normal tibial fracture healing was detected *via* RT and qPCR. RT reaction contained 500 ng RNA sample, which was divided into three parts, respectively. Total RNA in each group was diluted 10 times, and 3 μL RNA sample was taken for Polymerase Chain Reaction amplification. After that, the amplification level of the target gene was verified by 5% agarose gel electrophoresis. LabWorks 4.0 image acquisition and analysis software were adopted for quantification and data processing. To obtain reliable data, this experiment was repeated three times. Finally, changes in the relative expression level of lncRNAs were analyzed by the $2^{-\Delta\Delta Ct}$ method. Primer sequences used in this study were as follows: PCAT6, F: 5'-GAGGAACCTTCTCAACTG-3', R: 5'-CGTAGGGACCTGAACTAGGA-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Cell Culture

Human chondrocyte strain hFOB1.19 was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA, batch number:

12491015) containing 10% horse serum (Invitrogen, Carlsbad, CA, USA, batch number: 444262) and 5% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA, batch number: 16000-044), and maintained in an incubator containing 5% CO_2 at the constant temperature of 37°C.

Cell Transfection

LncRNA KCNQ1OT1 knockdown experiment was conducted using 3 different probes for lncRNA KCNQ1OT1 and negative control. Plasmids of pcDNA3-KCNQ1OT1 were transfected into HC-a cells to overexpress KCNQ1OT1 *in vitro*. Oligonucleotide sequences were designed by Exiqon's GapmeR algorithm, and its LNATM mode was as follows: (http://www.exiqon.com/ls/Pages/DDTSequenceInput.aspx? SkipCheck = true).

Cell Proliferation via Cell Counting Kit-8 (CCK-8)

Cells were first inoculated in 96-well plates. After cell adhesion reached 80%, they were synchronized for 12 h. Subsequently, the original culture medium was discarded. Total reaction volume of each well was 200 μL, and 6 wells were set for each sample. Briefly, 20 μL of Cell Counting Kit-8 reaction solution (Dojindo, Kumamoto, Japan) was added to each well, followed by incubation at 37°C in the dark for 2 h. The cells were vibrated on a micro vibrator for 10 min. Finally, A_{450} of each well was measured by a microplate reader.

Cell Apoptosis

Annexin V/propidium iodide (PI) double staining kit was applied to detect the apoptotic changes of PC12 cells after treatment. 5×10^5 cells were first digested with pancreatin and rinsed twice with Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA) at 4°C. After centrifugation, the cells were resuspended in 500 μL of staining buffer. Subsequently, 5 μL of Annexin V-FITC (fluorescein isothiocyanate) and 5 μL of PI staining solution were added for staining away from light at 37°C for 15 min. Finally, Guava flow cytometer (Luminex Corporation, Austin, TX, USA) was used for flow sample loading and detection.

Protein Expression Changes via Western Blotting

The cell lysate was prepared, and an appropriate amount of radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) was taken. Protease inhibitor phenylmethylsulfonyl fluoride (PMSF) was added and mixed evenly with RIPA (RIPA: PMSF=100: 1; Beyotime, Shanghai, China). After digestion with trypsin, the cells were collected and added with cell lysate to collect cell lysate products. Then, they were transferred into Eppendorf (EP; Eppendorf, Hamburg, Germany) tubes, followed by centrifugation at 4°C and 14000 rpm for 30 min. Protein supernatant was extracted, and the proteins were denatured after heating bath at 9°C for 10 min. After that, prepared protein samples were stored in a refrigerator at -80°C for use. The concentration of proteins was quantified using the bicinchoninic acid (BCA) kit (Pierce, Waltham, MA, USA). After quantification, protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel under the constant voltage (80 V) for 2.5 h. Subsequently, the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) by semi-dry transfer method. After immersed in Tris-Buffered Saline and Tween 20 (TBST; Sigma-Aldrich, St. Louis, MO, USA) containing 5% skim milk powder, the membranes were slowly shaken for 1 h. After sealing, the membranes were incubated with primary antibodies diluted with 5% skim milk powder overnight. On the next day, the membranes were incubated with the corresponding secondary antibody at room temperature for 2 h. The membranes were rinsed twice with TBST and once with Tris-Buffered Saline (with 10 min each time). Immunoreactive bands were detected using electrochemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA) reagent and exposed in a dark room. Finally, Adobe Photoshop software was adopted to analyze the relative expression of proteins.

Animal Model

A total of 36 adult healthy white rabbits (18 males and 18 females) from New Zealand with an average body mass of (1.8±0.5) kg were selected. All rabbits were kept in single cages, with free access to water. The rabbits were randomly divided into two groups, namely, the experimental group (n=18) and control group (n=18). All rabbits were first intravenously injected with 50 mg/kg pentobarbital sodium. After anesthesia, the left hind limb was sheared and disinfected. Meanwhile, the skin was surgically cut open to separate subcutaneous tissues and expose the tibia. Then, the

incomplete fracture of the middle tibia of rabbits was caused by a double-layer hacksaw, with a width of about 3 mm and the depth of about 1/3 of the tibia. The wound was sutured after the operation. After successful surgical modeling, rabbits in the experimental group were intravenously injected with KCNQ1OT1 adenovirus-short hairpin RNAs (shRNAs; 1×10^9 TU dissolved in 50 μL of saline solution) purchased from GenePharma (Shanghai, China) from the ear margin. After 8 weeks of modeling, X-ray (CR) was used to examine the fracture. The formation and healing of fractures and calluses were observed as well. In the 2nd, 4th, 6th and 8th week, rabbits in each group were anesthetized with 1% pentobarbital and 3 rabbits were killed. The fractured tibia was cut off, cleaned and frozen at -80°C for later use[11].

Determination of Alkaline Phosphatase (ALP) and Osteoprotegerin (OPG) in the Serum of Rabbits

5 mL of the blood sample was extracted from rabbits in the two groups at 2, 4, 6 and 8 weeks after fracture, respectively. Subsequently, the serum was obtained by centrifugation at 1000 rpm/min. The serum levels of ALP and OPG in rabbits were determined *via* enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 13.0 software (SPSS Inc., Chicago, IL, USA) was adopted for all statistical analysis. Experimental data were expressed as mean ± standard deviation. Student's *t*-test was used to compare the difference between the two groups. $p<0.05$ suggested that the difference was statistically significant.

Results

LncRNA KCNQ1OT1 was Significantly Down-regulated in Bone Tissues of Patients with Delayed Fracture Healing

To search for lncRNAs that played a key role in delayed fracture healing, lncRNA expression profiling microarray was used to investigate differentially expressed genes in bone tissues of 3 patients with delayed fracture healing and those with normal fracture healing. The results indicated that lncRNA KCNQ1OT1 was significantly down-regulated in bone tissues of patients with delayed fracture healing. Subsequently, the difference in lncRNA KCNQ1OT1 expression in bone tissues of 20 patients with delayed fracture healing and 20 patients with normal fracture healing was detected using qRT-PCR. The results manifested that lncRNA KCNQ1OT1 was remarkably down-regulated in bone tissues of patients with delayed fracture healing ($p<0.01$; Figure 1).

Knockdown of LncRNA KCNQ1OT1 Inhibited Tibial Fracture Healing in Rabbits

In the 2nd, 4th, 6th and 8th week after successful modeling, the levels of serum APL and OPG in lncRNA KCNQ1OT1 knockdown group were evidently reduced when compared with those of the control group, with statistically significant differences ($p<0.05$). Meanwhile, micro-CT scanning results demonstrated that bone trabecular growth indexes (including bone volume density and bone trabecular number) in lncRNA KCNQ1OT1 knockdown group were significantly lower than those of the control group, showing statistically significant differences ($p<0.05$; Figure 2).

LncRNA KCNQ1OT1 Activated the Wnt/β-Catenin Signaling Pathway

GSEA analysis of gene expression profiling data revealed that the Wnt/β-catenin signaling pathway in bone tissues of patients with normal fracture healing was notably up-regulated when compared with that of patients with delayed fracture healing ($p<0.01$). Furthermore, the correlation between the expression levels of lncRNA KCNQ1OT1 and β-catenin mRNAs in tissues of fracture patients was analyzed using Pearson's correlation coefficient. It was found that the expression level of lncRNA KCNQ1OT1 was positively correlated with the expression level of β-catenin ($p<0.01$, $r=0.53$). Subsequently, the influence of lncRNA KCNQ1OT1 expression on the Wnt/β-catenin signaling pathway was investigated by *in vitro* experiments. Western blotting results confirmed that compared with the control group, overexpression of lncRNA KCNQ1OT1 remarkably promoted the expressions of β-catenin and low-density lipoprotein receptor-related protein 5 (LRP5) in the Wnt/β-catenin signaling pathway. Meanwhile, KCNQ1OT1 overexpression markedly increased the protein expression levels of lymphatic enhancement factor 1 (LEF1) and c-Myc in the downstream of the Wnt/β-catenin signaling pathway, whereas significantly reduced the expression levels of

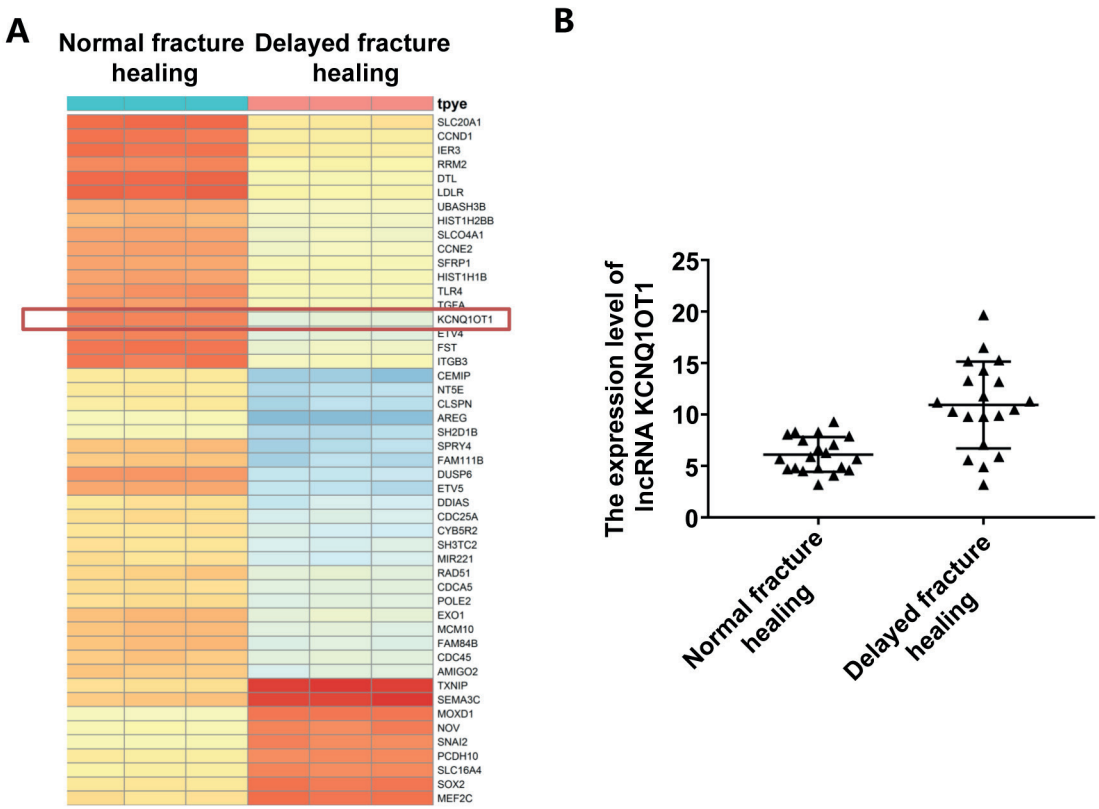

Figure 1. LncRNA KCNQ1OT1 is markedly down-regulated in bone tissues of patients with delayed fracture healing. ***A,*** LncRNA expression profiling microarray data of patients with normal fracture healing and those with delayed fracture healing (p<0.01, fold change >2). ***B,*** Difference in the expression level of lncRNA KCNQ1OT1 in bone tissues of patients with delayed fracture healing and those with normal fracture healing compared by qRT-PCR (p<0.01).

Dickkopf-1 (DKK1) and sclerostin (SOST) in the Wnt/β-catenin signaling pathway. Besides, compared with the control group, knocking down lncRNA KCNQ1OT1 evidently inhibited the protein expression levels of β-catenin, LRP5 and its downstream protein LEF1. However, KCNQ1OT1 down-regulation remarkably increased the expression levels of inhibitor proteins DKK1 and SOST of the Wnt/β-catenin signaling pathway. The results were shown in Figure 3.

LncRNA KCNQ1OT1 Promoted HC-a Cell Proliferation by Activating the Wnt/β-Catenin Signaling Pathway

In this study, lncRNA KCNQ1OT1 was knocked down or overexpressed in HC-a cells to investigate the effect of lncRNA KCNQ1OT1 on HC-a cell proliferation. CCK-8 results showed that compared with the control group, knocking down lncRNA KCNQ1OT1 significantly reduced the proliferation rate of HC-a cells (p<0.01). However, overexpression of lncRNA KCNQ1OT1 markedly increased the proliferation rate of HC-a cells (p<0.01). At 24 h, CFSE staining results of HC-a cells revealed that compared with the control group, the proliferation rate of cells in lncRNA KCNQ1OT1 overexpression group was significantly elevated, whereas was markedly reduced in lncRNA KCNQ1OT1 overexpression group (Figure 4).

LncRNA KCNQ1OT1 Inhibited HC-a Cell Apoptosis by Activating the Wnt/β-Catenin Signaling Pathway

In this work, lncRNA KCNQ1OT1 was knocked down or overexpressed in HC-a cells to investigate the effect of lncRNA KCNQ1OT1 on HC-a cell apoptosis. Annexin V-PI staining results that compared with the control group, the apoptosis rate of cells in lncRNA KCNQ1OT1 overexpression group was notably reduced. However, cell apoptosis was markedly up-regulated in the lncRNA KCNQ1OT1 knockdown group.

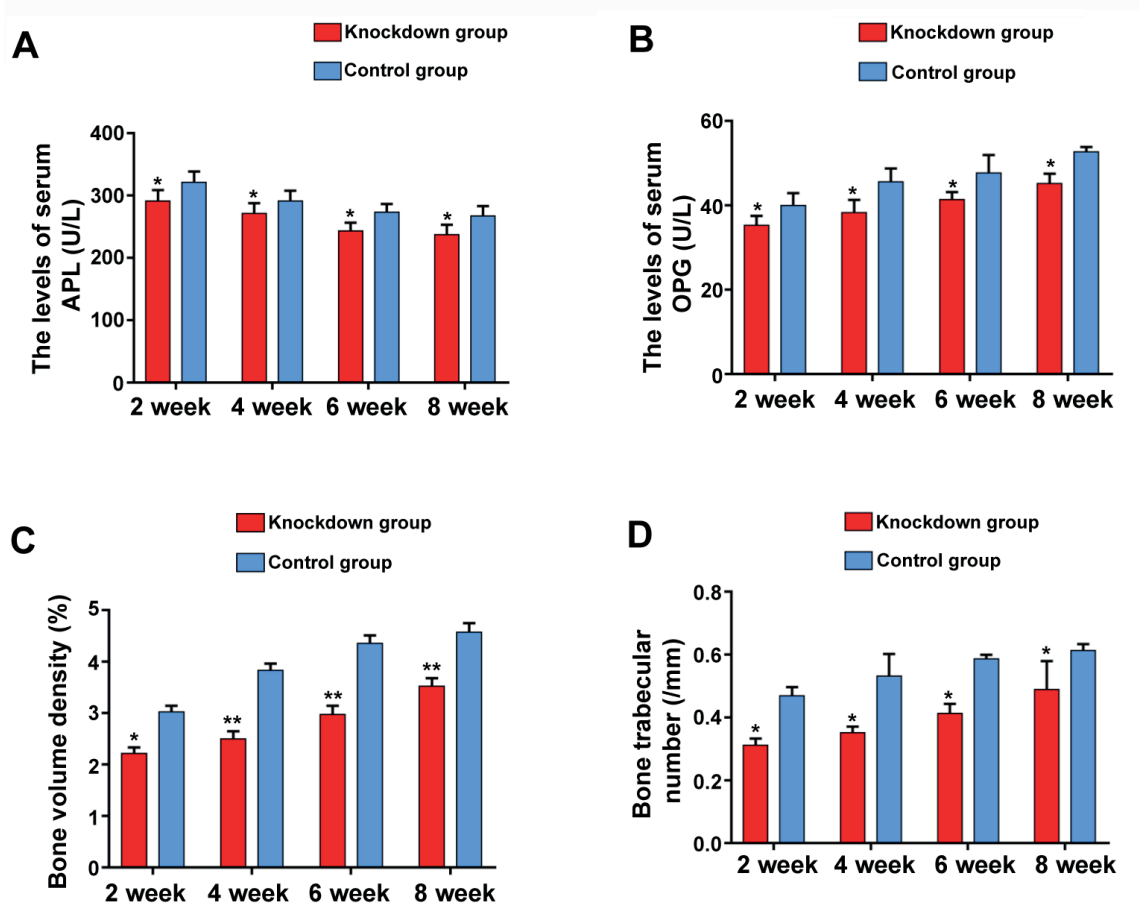

Figure 2. Effect of lncRNA KCNQ1OT1 expression on tibial fracture healing in rabbits.

Discussion

Delayed fracture healing is a common complication of bone defect treatment. At present, it is difficult to treat this disease clinically. Meanwhile, patients are often faced with amputation risks[12]. Bone tissue has a strong self-repairing ability. Bone defect site can be completely replaced by new bone tissues, and its original structure and function are finally restored[13]. Current epidemiological investigation results have indicated that the incidence rate of delayed fracture healing or non-healing due to trauma is increasing year by year, accounting for about 3% of total incidence rate of bone diseases. A large number of research has revealed that lncRNAs participate in varying key biological processes. Moreover, lncRNAs have been confirmed to take part in the biological processes of fracture healing. Therefore, searching for key biomolecules in the process of fracture healing and exploring key regulatory signaling pathways will provide important therapeutic bases and new drug targets for improving the state of delayed fracture healing and non-healing.

In this work, gene expression profiling microarray and qRT-PCR results demonstrated that lncRNA KCNQ1OT1 was markedly reduced in bone tissues of patients with delayed fracture healing. Furthermore, the rabbit model of tibial fractures was successfully established *in vivo*. The results illustrated that knocking down lncRNA KCNQ1OT1 evidently inhibited the process of fracture healing in rabbits in a time-dependent manner. The above results indicated that lncRNA KCNQ1OT1 exerted a crucial effect on fracture healing. In the current study, the results showed that lncRNA KCNQ1OT1 promoted the proliferation and inhibited apoptosis of bone cells by activating the Wnt/β-catenin signaling pathway, which was supported by the following evidences: (1) GSEA

of gene expression profiling microassay data manifested that the Wnt/β-catenin signaling pathway was markedly elevated in bone tissues of patients with normal fracture healing compared with that of patients with delayed fracture healing (p<0.01). Pearson's correlation coefficient analysis indicated that the expression level of lncRNA KCNQ1OT1 was positively correlated with the expression level of β-catenin mRNAs to a moderate degree in 40 fracture patients (p<0.01, r=0.53). (2) Overexpression and knockdown experiments demonstrated that overexpression of lncRNA KCNQ1OT1 notably promoted the activation of the Wnt/β-catenin signaling pathway. However, knockdown of lncRNA KCNQ1OT1 significantly inhibited the protein expression levels of β-catenin, LRP5 and its downstream protein LEF1. Moreover, KCNQ1OT1 knockdown remarkably increased the protein expression levels of inhibitors (including DKK1 and SOST) of the Wnt/β-catenin signaling pathway. (3) Compared with cells in the control group, overexpression of lncRNA KCNQ1OT1 evidently promoted cell proliferation and inhibit cell apoptosis. However, the application of the inhibitor ICG-001 of the Wnt/β-catenin pathway could reverse cell proliferation and apoptosis. With Pull-down assay, Gao et al[7] have found that lncRNA KCNQ1OT1 can directly interact with β-catenin, thus inhibiting the degradation of β-catenin and further supporting the results of this study. Therefore, the Wnt/β-catenin signaling pathway plays a key role in fracture healing induced by lncRNA KCNQ1OT1.

Wnt/β-catenin signaling pathway, also known as the classical Wnt pathway, is a highly conserved signaling pathway in evolution[14]. It can inhib-

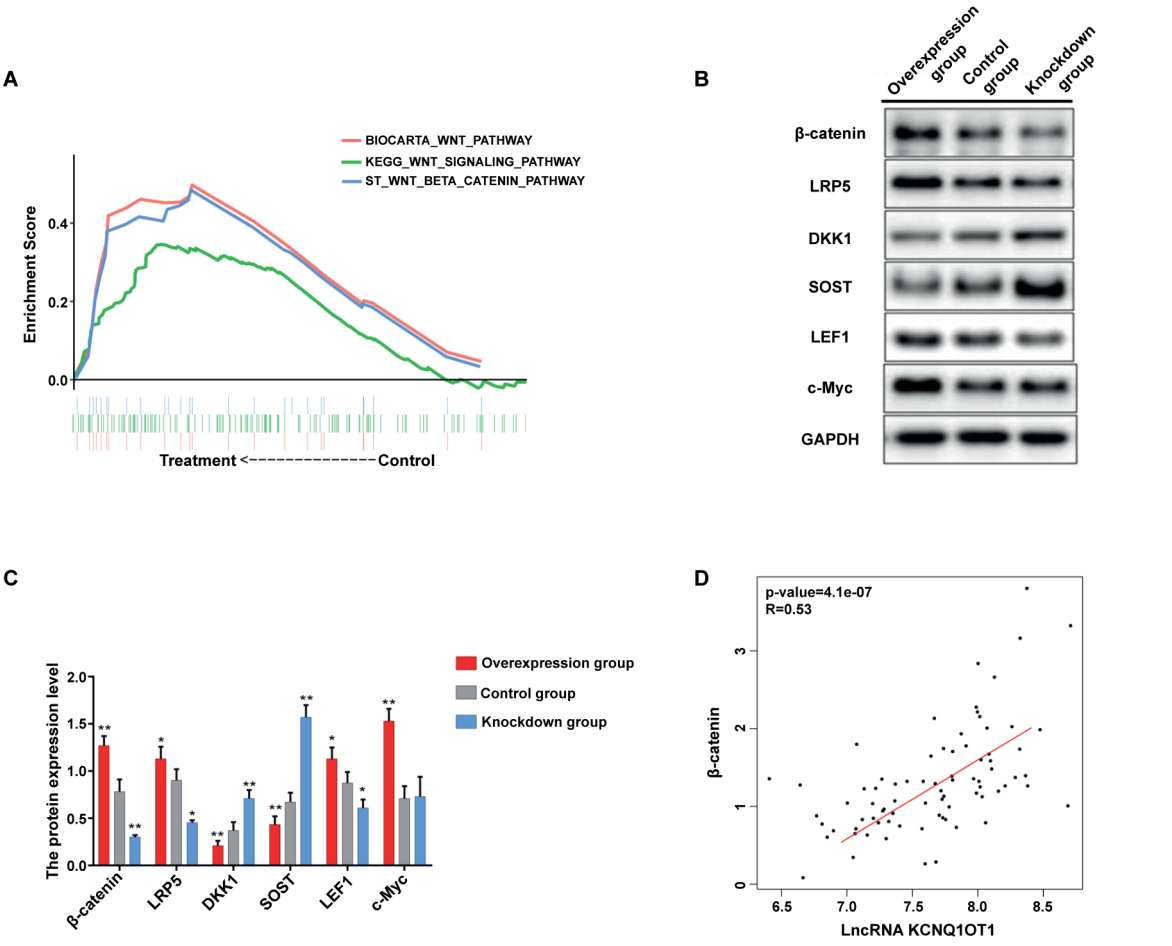

Figure 3. LncRNA KCNQ1OT1 activates the Wnt/β-catenin signaling pathway. ***A,*** GSEA analysis results of gene expression profiling microassay for patients with delayed fracture healing and those with normal fracture healing. ***B,*** Influence of the expression level of lncRNA KCNQ1OT1 on the Wnt/β-catenin signaling pathway detected *via* Western blotting. ***C,*** Quantitative analysis of protein expression level. **: p<0.01, *: p<0.05. ***D,*** Correlation between the expression levels of lncRNA KCNQ1OT1 and β-catenin in tissues of fracture patients analyzed using Pearson's correlation coefficient (p<0.01, r=0.53).

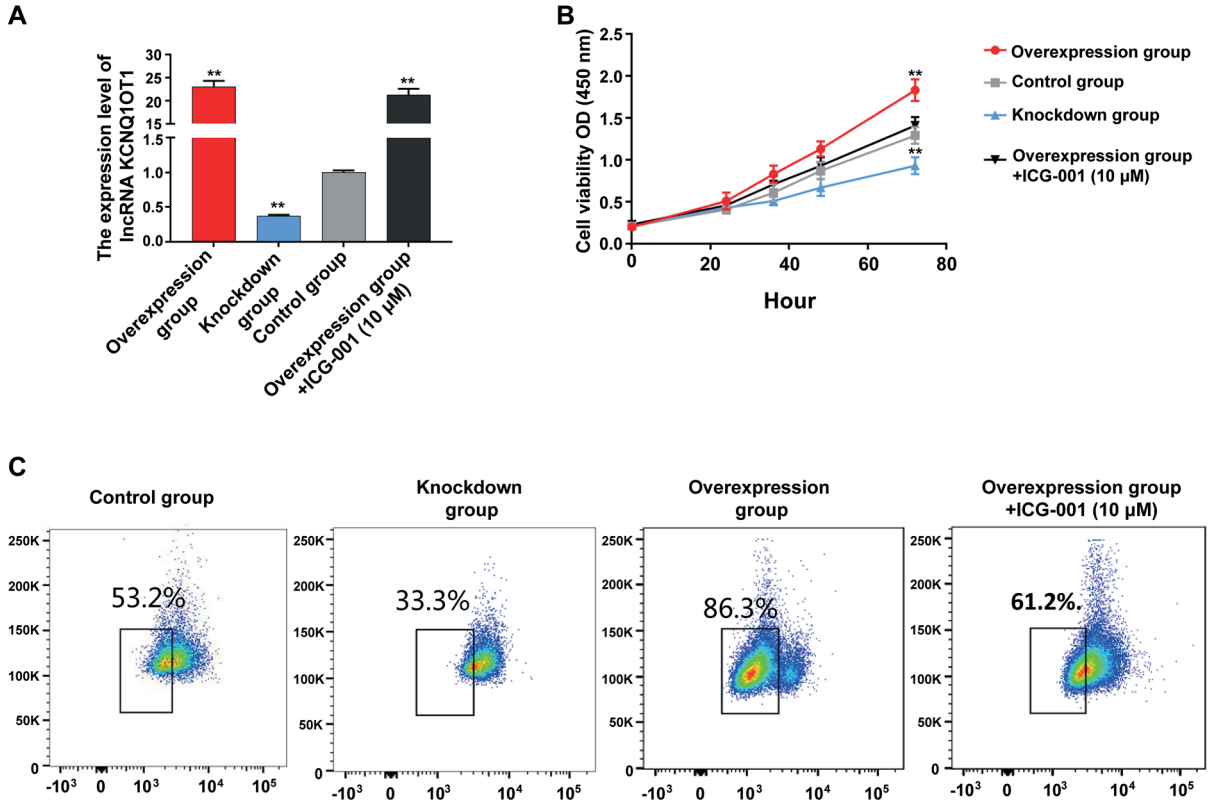

Figure 4. LncRNA KCNQ1OT1 promotes HC-a cell proliferation by activating the Wnt/β-catenin signaling pathway. ***A***, Comparison of the expression levels of lncRNA KCNQ1OT1 among overexpression group, knockdown group and control group ($^{**}p<0.01$). ***B***, Proliferation curves of three groups of cells detected *via* CCK-8 assay ($^{**}p<0.01$). ***C***, Influence of the expression level of lncRNA KCNQ1OT1 on HC-a cell proliferation *via* CFSE.

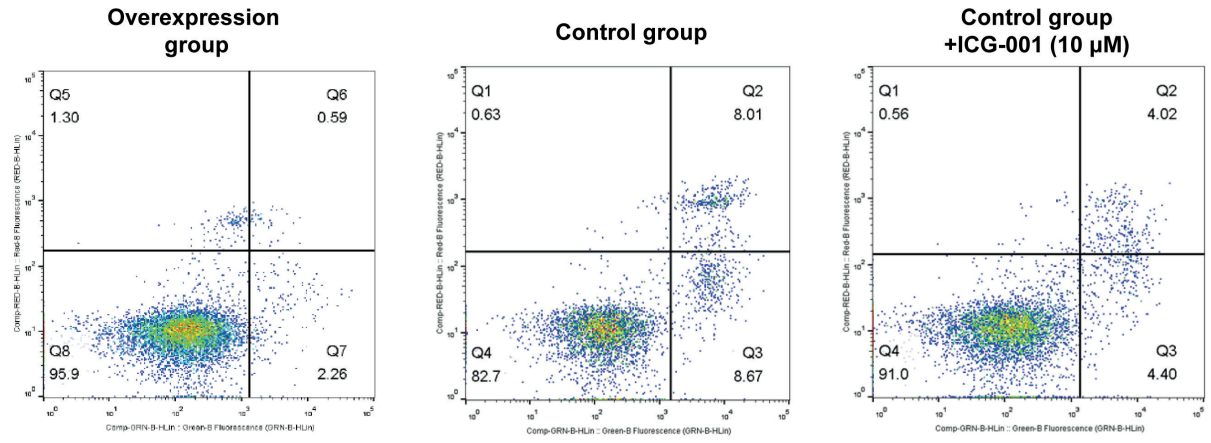

Figure 5. LncRNA KCNQ1OT1 inhibits HC-a cell apoptosis by activating the Wnt/β-catenin signaling pathway.

it phosphorylation and degradation of β-catenin, thus enabling β-catenin to accumulate in the cytoplasm and transfer to the nucleus. β-catenin combines with transcription factors in the nucleus and regulates the expression of multiple target genes. Ligands of the Wnt signaling pathway include Wnt4, Wnt5a, Wnt5b, Wnt10b, Wnt11 and Wnt13. Meanwhile, receptors of the Wnt signal-

ing pathway include Fzd1, 2, 4, 5 and 9, Lrp5 and Lrp6. Runx2 and c-Myc are target genes of β-catenin and Wnt. Current researches have shown that fracture healing in mice can be improved by activating the Wnt signaling pathway through the administration of classical Wnt agonists or antibodies against classical Wnt inhibitors (DKK1 and SOST)[15]. The activation mechanism of the Wnt/β-catenin signaling pathway includes attracting MSCs to the injured site, directionally controlling MSCs to differentiate into osteoblasts instead of chondrocytes, as well as enhancing bone formation and reducing bone resorption that is conducive to fracture healing[16,17].

Conclusions

We showed that lncRNA KCNQ1OT1 plays a crucial role in delayed fracture healing. It can induce cell proliferation and inhibit cell apoptosis by activating the Wnt/β-catenin signaling pathway. Therefore, KCNQ1OT1 may be used as a biomarker to predict the occurrence of delayed fracture healing. Our findings may also contribute to the understanding of the mechanism of delayed fracture healing.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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