Advances in Rheumatology

RESEARCH Open Access

Long non-coding RNA KCNQ1OT1 promotes cell viability and migration as well as inhibiting degradation of CHON-001 cells by regulating miR-126-5p/TRPS1 axis

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Abstract

Background: Osteoarthritis (OA) is defined as a degenerative disease. Pivotal roles of long non-coding RNA (lncRNAs) in OA are widely elucidated. Herein, we intend to explore the function and molecular mechanism of lncRNA KCNQ1OT1 in CHON-001 cells.

Methods: Relative expression of KCNQ1OT1, miR-126-5p and TRPS1 was determined by quantitative real-time polymerase chain reaction (qRT-PCR). Cell viability was examined by MTT assay. The migratory ability of chondrocytes was assessed by transwell assay. Western blot was used to determine relative protein expression of collagen II, MMP13 and TRPS1. Dual-luciferase reporter (DLR) assay was applied to test the target of lncRNA KCNQ1OT1 or miR-126-5p.

Results: Relative expression of KCNQ1OT1 and TRPS1 was reduced, whereas miR-126-5p was augmented in cartilage tissues of post-traumatic OA patients compared to those of subjects without post-traumatic OA. Increased KCNQ1OT1 or decreased miR-126-5p enhanced cell viability and migration, and repressed extracellular matrix (ECM) degradation in CHON-001 cells. MiR-126-5p was the downstream target of KCNQ1OT1, and it could directly target TRPS1. There was an inverse correlation between KCNQ1OT1 and miR-126-5p or between miR-126-5p and TRPS1. Meantime, there was a positive correlation between KCNQ1OT1 and TRPS1. The promoting impacts of KCNQ1OT1 on cell viability and migration as well as the suppressive impact of KCNQ1OT1 on ECM degradation were partially abolished by miR-126-5p overexpression or TRPS1 knockdown in CHON-001 cells.

Conclusions: Overexpression of KCNQ1OT1 attenuates the development of OA by sponging miR-126-5p to target TRPS1. Our findings may provide a possible therapeutic strategy for human OA in clinic.

Keywords: Post-traumatic osteoarthritis, KCNQ1 overlapping transcript 1, miR-126-5p, Tricho-rhino-phalangeal syndrome type I

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Introduction

Osteoarthritis (OA) is a degenerative bone and joint disease, which is featured by destruction of joint matrix and the decrease of articular chondrocytes [1, 2]. Its clinical symptoms include swelling, stiffness and arthralgia [3]. As previously reported, OA can be caused by diverse factors, such as genetic predisposition, failure of nutrient supply, abnormal mechanical loading and trauma [1]. Currently, drug treatment, stem cell treatment, electromagnetic therapy and acupuncture therapy have been clinically applied for OA therapy [4–7]. However, the treatment effect is unsatisfactory. Thence, it is imperative to explore more effective targets for OA management.

Recent years, the crucial roles of long non-coding RNAs (lncRNAs) in OA have been gradually recognized [8–10]. For instance, MALAT1 and MFI2-AS1 are deeply involved in chondrocytes proliferation and extracellular matrix (ECM) degradation in OA [8, 9]. Similarly, TUG1 can facilitate ECM degradation of chondrocytes in OA [10]. Notably, lncRNA KCNQ1 overlapping transcript 1 (KCNQ1OT1), located in the KCNQ1 locus at 11p15.5 [11], has been reported to play important role in the proliferation and differentiation of chondrocytes in fracture or osteolysis [12, 13]. However, the role and mechanism of lncRNA KCNQ1OT1 in OA are not elucidated.

It is well known that lncRNAs participate in post-transcriptional regulation by binding to microRNAs (miRNAs) [14]. Ample evidence has revealed that miRNAs are critical regulators in the pathogenesis of OA [15], such as miR-335-5p [16] and miR-140 [17]. More importantly, miR-126 is also uncovered to possess a regulatory role in OA [18, 19]. For example, inhibition of miR-126 promotes the viability and migration of chondrocytes in a cell model of OA [18]. The viability of chondrocytes is enhanced by down-regulation of miR-126 [19]. In addition, miR-126 is revealed to be a target of lncRNA DNM3OS to regulate OA progression [19]. Nonetheless, the regulatory effect of lncRNA KCNQ1OT1 on miR-126-5p in OA has not been explored.

Tricho-rhino-phalangeal syndrome type I (TRPS1) is known as an oncogene to promote cell proliferation and metastasis in several types of human cancers, such as breast cancer [20], osteosarcoma [21], and colon cancer [22]. In recent years, the promoting role of TRPS1 in chondrocytes differentiation and proliferation has attracted increasing attention [23, 24]. Wuelling et al. found that loss of TRPS1 represses the differentiation of chondrocytes via Wnt5a [23]. Suemoto et al. demonstrated that overexpression of TRPS1 facilitates chondrocytes proliferation by reduction the level of Stat3 [24]. However, studies of TRPS1 effects on OA are limited.

Here, the roles of lncRNA KCNQ1OT1 and miR-126-5p were examined in CHON-001 cells. To further explore the regulatory mechanism of lncRNA KCNQ1OT1, the regulatory relation among KCNQ1OT1, miR-126-5p and TRPS1 was tested in CHON-001 cells. This investigation provided a new theoretical reference for OA treatment.

Materials and methods

Patients

The OA cartilage tissues were obtained from knees of post-traumatic OA (PTOA) patients (14 males and 9 females, an average age of 56.58 years) who underwent total knee arthroplasty. Normal cartilage tissues were collected from subjects without PTOA (8 males and 8 females, an average age of 51.66 years) who underwent the amputation. All samples were collected from our hospital between March 2017 and December 2019. The tissues samples were used to detect the expression levels of KCNQ1OT1, miR-126-5p, and TRPS1, as well as the correlations among them. The current study was approved by the ethics committees of our hospital, and all participants had signed written informed consents.

Cell culture

A human chondrocyte cell line (CHON-001) was bought from American Type Culture Collection (ATCC; Manassas, Virginia, USA). CHON-001 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Waltham, MA, USA) supplemented with 0.1 mg/mL G-418 (Gibco) and 10% fetal bovine serum (FBS; Gibco), and maintained in an incubator with a temperature of 37 °C and 5% CO_2.

Cell transfection

The pcDNA3.1-negative control (NC), pcDNA3.1-KCNQ1OT1 (containing full-length of KCNQ1OT1), short hairpin (sh)-NC, sh-KCNQ1OT1, mimics NC, miR-126-5p mimics, inhibitor NC, miR-126-5p inhibitor, sh-TRPS1 were bought from RiboBio (Guangzhou, China). Then these oligonucleotides and plasmids were transfected into CHON-001 cells through Lipofectamine3000 (Invitrogen, Carlsbad, CA, USA) for 48 h. Afterwards, the transfected CHON-001 cells were used to perform the functional experiments of KCNQ1OT1/miR-126-5p/TRPS1 axis in vitro.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA from cartilage tissues and CHON-001 cells was extracted using TRIzol® reagent (Invitrogen), and RNA concentration was detected by NanoDropND-1000 spectrophotometer. After that, extracted RNA was converted to complementary DNA (cDNA) via a PrimeScript™ RT reagent kit (Takara, Beijing, China). The cDNA was

used for performing qRT-PCR according to instructions of a SYBR Green PCR kit (Takara). The reaction procedures were shown as follows: 95 °C for 20 s, 35 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 10 s. All primers (Table 1) were bought from Sangon Biotech (Shanghai, China). Finally, relative expression of KCNQ1OT1, miR-126-5p and TRPS1 was calculated via the $2^{-\Delta\Delta Ct}$ method. U6 and GADPH were used as internal references.

3-(4, 5-Dimethyl-2-Thiazolyl)-2, 5-Diphenyl-2-H-tetrazolium bromide (MTT) assay

CHON-001 cells (6×10^3/well) were seeded in 96-well plates, and then reacted with MTT (10 μL). After 4 h of reaction, and dimethyl sulfoxide (150 μL) were added into each well for dissolving formazan crystals. Cell viability was assessed by measuring the optical density (OD) at 450 nm using a microplate reader (BMG LABTECH, Durham, NC, USA).

Dual Luciferase Reporter (DLR) assay

The 3′-UTR sequence of KCNQ1OT1 or TRPS1 containing miR-126-5p binding sites was cloned into a pGL3 Basic Vector (Promega, Madison, WI) to construct KCNQ1OT1 wt or TRPS1 wt. KCNQ1OT1 mut or TRPS1 mut was obtained by mutating binding sites of miR-126-5p and then introduced into the pGL3 Basic Vector (Promega). Then CHON-001 cells were transfected with above plasmids along with mimics NC/miR-126-5p mimics. Following 48 h of transfection, Firefly and Renilla luciferase activity was examined through a dual-luciferase reporter assay system (Biotek, Winooski, VT, USA). Relative luciferase activity was defined as the ratio of Firefly luciferase activity and Renilla luciferase activity.

Western blot

Total proteins were isolated from CHON-001 cells using RIPA lysis (Beyotime, Shanghai, China), and the concentration of proteins was measured through a bicinchoninic acid kit (Pierce, Waltham, MA, USA). Next, protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, followed by transferring onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After being blocked with 5% skim milk, the membranes were incubated with the following primary antibodies overnight at 4 °C: anti-TRPS1 (1:1000, ab227867, Abcam), anti-collagen II (1:2000, ab188570, Abcam), anti-MMP13 (1:1000, ab51072, Abcam) and anti-α-tubulin (1:2000, ab52866, Abcam). After the membranes were washed with tris buffered saline Tween (TBST), the membranes were nurtured with the secondary antibody (1:10,000, ab205718, Abcam) at 37 °C for 2 h. Finally, the immune-reactivity was visualized using electrochemiluminescence (Thermo Fisher Scientific, Shanghai, China), and relative protein expression of MMP13, TRPS1 and collagen II over α-tubulin was quantified via Gel-pro Analyzer software (Media Cybernetics, Maryland, USA).

Transwell assay

For determining the migratory ability of CHON-001 cells, transwell assay was carried out with the 24-well transwell chamber (8-μm pore size; Corning, Cambridge, USA). CHON-001 cells (6×10^5 cells) in FBS-free medium were plated into the upper layer of the transwell chamber. Subsequently, the lower chamber was filled with 600 μL DMEM including 10% FBS. After incubation for 24 h, migratory cells on the lower chamber were fixed in 4% paraformaldehyde for 15 min and stained with 0.5% crystal violet for 10 min. The numbers of migratory cells were counted under a Leica microscope (Leica Microsystems, Wetzlar, Germany). The percentage of migration was calculated as the following formula: (the numbers of migratory cells in the treat group)/ (the numbers of migratory cells in the control group) × 100%.

Statistical analysis

Data were analyzed by the SPSS 22.0 software (IBM, Armonk, NY, USA). All results were displayed as the mean ± standard deviation. One-way ANOVA was utilized for multiple comparisons, followed by Tukey's post hoc test. Student's t-test was applied to compare differences between two groups. A value of $P < 0.05$ was regarded as statistically significant.

Results

LncRNA KCNQ1OT1 promotes cell viability and migration, and represses ECM degradation in CHON-001 cells

In order to probe the function of lncRNA KCNQ1OT1 in OA, the expression of KCNQ1OT1 was firstly

Table 1 Primers for qRT-PCR

Gene	Forward	Reverse
KCNQ1OT1	5′-CTTTGCAGCAACCTCCTTGT-3′	5′-TGGGGTGAGGGATCTGAA-3′
MiR-126-5p	5′-GCGCGCATTATTACTTTTGG-3′	5′-TGGTGTCGTGGAGTCG − 3′
TRPS1	5′-CAAATCTCAGGCCTGAGTGA − 3′	5′-GTGAAGAGCTGATATCCTGCAG-3′
U6	5′-CTCGCTTCGGCAGCACA-3′	5′-AACGCTTCACGAATTTGCGT-3′
GAPDH	5′-CAAAGGTGGATCAGATTCAAG-3′	5′-GGTGAGCATTATCACCCAGAA-3′

determined in PTOA patients. We found that KCNQ1OT1 expression was lower in the PTOA group than that in the control group ($P < 0.001$, Fig. 1A). Subsequently, lncRNA KCNQ1OT1 was overexpressed or silenced in CHON-001 cells. As expected, KCNQ1OT1 expression was evidently diminished by addition of sh-KCNQ1OT1, and distinctly boosted by addition of pcDNA3.1-KCNQ1OT1 in CHON-001 cells (all $P < 0.001$, Fig. 1B). Then functional experiments were performed. Results from MTT and transwell assays displayed that the viability ($P < 0.001$, Fig. 1C) and migratory ability of CHON-001 cells ($P < 0.01$, Fig. 1D) were enhanced when KCNQ1OT1 was overexpressed, whereas silencing of KCNQ1OT1 resulted in opposite results. Moreover, data from western blot indicated that up-regulation of KCNQ1OT1 elevated relative protein expression of the ECM protein (collagen II) ($P < 0.01$, Fig. 1E), whereas reduced relative protein expression of the cartilage-degrading enzyme (MMP13) in CHON-001 cells ($P < 0.001$, Fig. 1E), thereby repressing the ECM

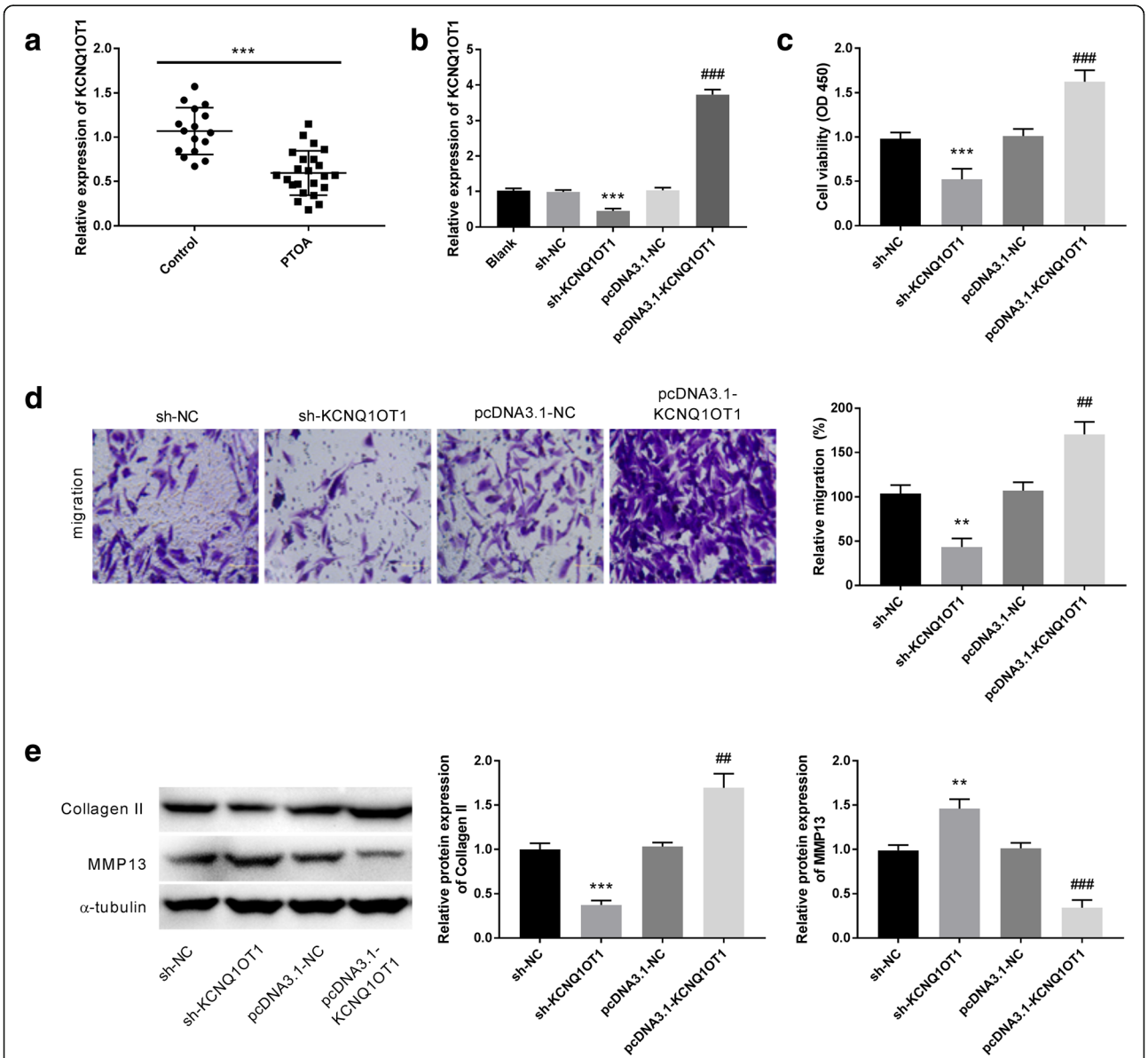

Fig. 1 LncRNA KCNQ1OT1 promotes cell viability and migration, and suppresses ECM degradation in CHON-001 cells. **a** Relative expression of KCNQ1OT1 was detected by qRT-PCR in cartilage tissues of PTOA patients and normal cartilage tissues. $^{***}P < 0.001$, vs. Control. **b** Relative expression of KCNQ1OT1 was determined by qRT-PCR in CHON-001 cells. $^{***}P < 0.001$, vs. sh-NC. $^{###}P < 0.001$, vs. pcDNA3.1-NC. **c** The cell viability was determined by MTT assay in CHON-001 cells. $^{***}P < 0.001$, vs. sh-NC. $^{###}P < 0.001$, vs. pcDNA3.1-NC. **d** Relative migration was assessed by transwell assay in CHON-001 cells. $^{**}P < 0.01$, vs. sh-NC. $^{##}P < 0.01$, vs. pcDNA3.1-NC. **e** Relative protein expression of collagen II and MMP13 was determined by western blot in CHON-001 cells. $^{**}P < 0.01$, $^{***}P < 0.001$, vs. sh-NC. $^{##}P < 0.01$, $^{###}P < 0.001$, vs. pcDNA3.1-NC

degradation of CHON-001 cells. In contrast to KCNQ1OT1 up-regulation, KCNQ1OT1 down-regulation made opposite effects on the protein expression of collagen II and MMP13 in CHON-001 cells.

LncRNA KCNQ1OT1 can directly bind to miR-126-5p

For exploring the downstream mechanism by which KCNQ1OT1 affected CHON-001 cells, we searched for targeting miRNAs of KCNQ1OT1 by starbase. Interestingly, we observed some complementary bases between miR-126-5p and KCNQ1OT1 (Fig. 2A). Meantime, DLR assay was used to verify the interaction of miR-126-5p and KCNQ1OT1. As shown in Fig. 2B, addition of miR-126-5p mimics markedly decreased relative luciferase activity of KCNQ1OT1 wt compared to addition of mimics NC ($P < 0.01$), while it failed to affect relative luciferase activity of KCNQ1OT1 mut in CHON-001 cells, confirming the direct binding between miR-126-5p and KCNQ1OT1 (Fig. 2B). Moreover, we discovered that relative expression of miR-126-5p was greatly boosted by knockdown of KCNQ1OT1 but reduced by overexpression of KCNQ1OT1 in CHON-001 cells (all $P < 0.001$, Fig. 2C). MiR-126-5p expression was higher in the PTOA group relative to that in the control group ($P < 0.001$, Fig. 2D). There was an inverse correlation between KCNQ1OT1 expression and miR-126-5p expression in cartilage tissues of PTOA patients ($P = 0.0171$, $R^2 = 0.2422$, Fig. 2E).

Decreased expression of miR-126-5p enhances the viability and migratory ability but represses ECM degradation in CHON-001 cells

Afterwards, to examine whether miR-126-5p made impacts on CHON-001 cells, CHON-001 cells were transfected with miR-126-5p mimics, miR-126-5p inhibitor and corresponding controls. As manifested in Fig. 3A, miR-126-5p mimics caused notable up-regulation of miR-126-5p, and miR-126-5p inhibitor led to distinct down-regulation of miR-126-5p in CHON-001 cells (all $P < 0.001$). Functionally, it was found that the cell viability ($P < 0.001$, Fig. 3B) and migratory ability ($P < 0.01$, Fig. 3C) were enhanced by miR-126-5p inhibitor, and reduced by miR-126-5p mimics in CHON-001 cells. Relative protein expression of collagen II was decreased by miR-126-5p mimics but elevated by miR-126-5p inhibitor in CHON-001 cells (all $P < 0.001$,

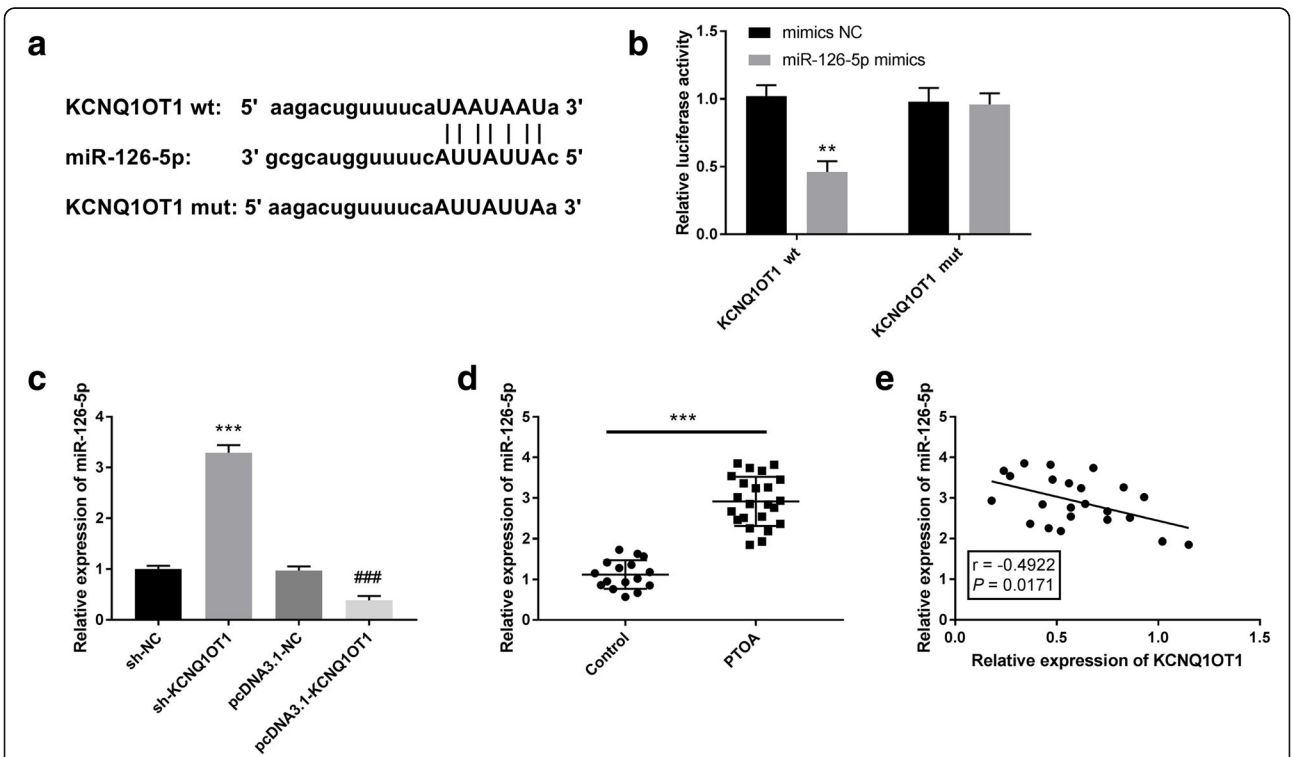

Fig. 2 LncRNA KCNQ1OT1 can directly bind to miR-126-5p. **a** The binding sequences between KCNQ1OT1 and miR-126-5p were predicted by starbase. **b** The interaction between KCNQ1OT1 and miR-126-5p in CHON-001 cells was verified by dual-luciferase reporter (DLR) assay. $^{**}P < 0.01$, vs. mimics NC. **c** Relative expression of miR-126-5p was detected by qRT-PCR in CHON-001 cells. $^{***}P < 0.001$, vs. sh-NC. $^{###}P < 0.001$, vs. pcDNA3.1-NC. **d** Relative expression of miR-126-5p was detected by qRT-PCR in cartilage tissues of PTOA patients and normal cartilage tissues. $^{***}P < 0.001$, vs. Control. **e** The correlation between KCNQ1OT1 and miR-126-5p was analyzed by Pearson's correlation analysis in cartilage tissues of PTOA patients. $P = 0.0171$. $R^2 = 0.2422$

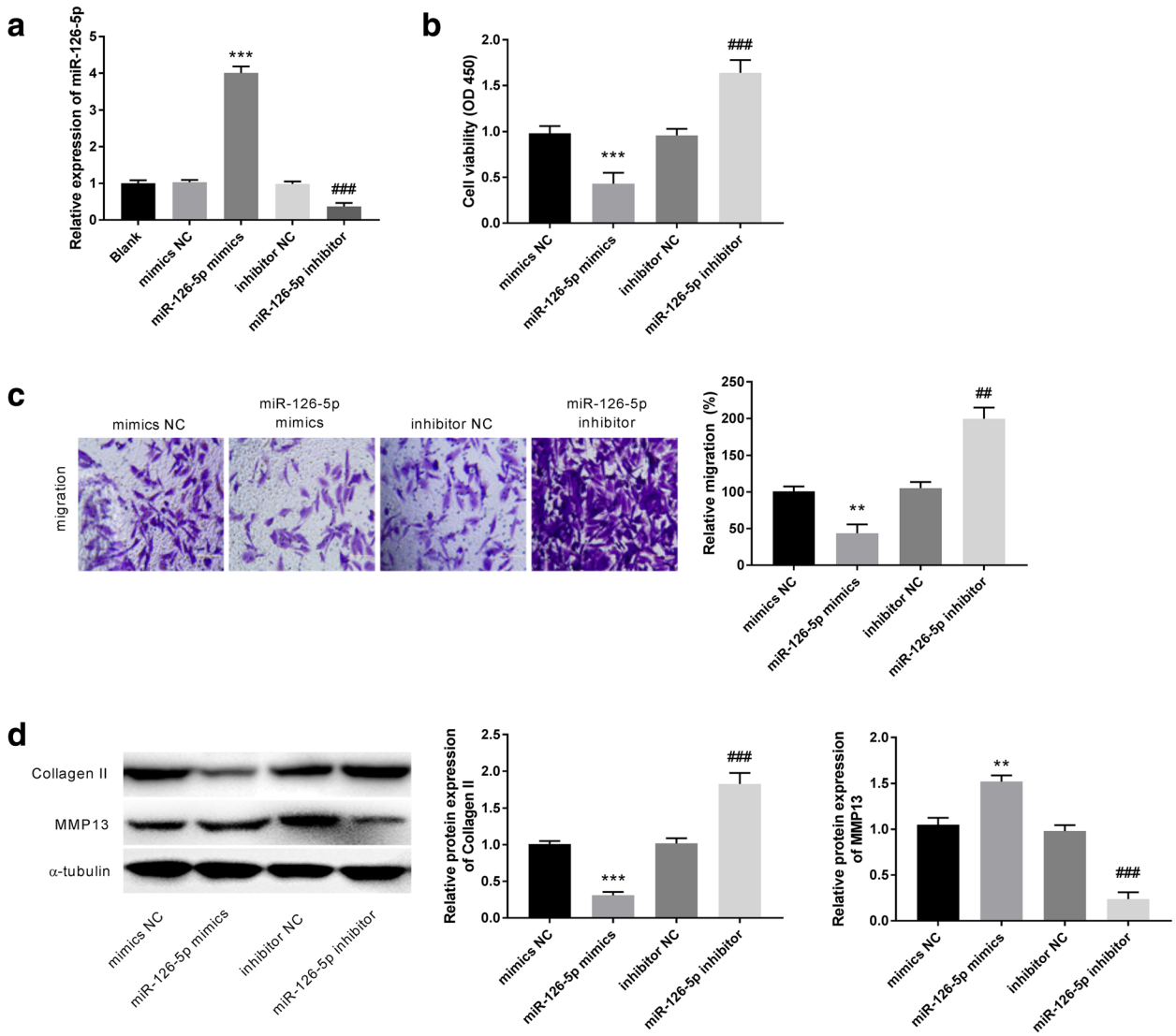

Fig. 3 Decreased expression of miR-126-5p enhances the viability and migratory ability of chondrocytes, and represses ECM degradation in CHON-001 cells. **a** Relative expression of miR-126-5p was determined by qRT-PCR in CHON-001 cells. $^{***}P < 0.001$, vs. mimics NC. $^{\#\#\#}P < 0.001$, vs. inhibitor NC. **b** The cell viability was determined by MTT assay in CHON-001 cells. $^{***}P < 0.001$, vs. mimics NC. $^{\#\#\#}P < 0.001$, vs. inhibitor NC. **c** Relative migration was assessed by transwell assay in CHON-001 cells. $^{**}P < 0.01$, vs. mimics NC. $^{\#\#}P < 0.01$, vs. inhibitor NC. **d** Relative protein expression of collagen II and MMP13 was determined by western blot in CHON-001 cells. $^{**}P < 0.01$, $^{***}P < 0.001$, vs. mimics NC. $^{\#\#\#}P < 0.001$, vs. inhibitor NC

Fig. 3D). Meantime, relative protein expression of MMP13 was boosted by miR-126-5p mimics ($P < 0.01$) but reduced by miR-126-5p inhibitor ($P < 0.001$) in CHON-001 cells (Fig. 3D).

TRPS1 was targeted by miR-126-5p

After that, target genes of miR-126-5p were predicted with starbase. It was uncovered that TRPS1 possessed binding regions of miR-126-5p (Fig. 4A). Meanwhile, the result of DLR displayed that relative luciferase activity of CHON-001 cells was evidently reduced after co-transfection of miR-126-5p mimics and TRPS1 wt ($P < 0.01$), while showed no significant change after co-transfection of miR-126-5p mimics and TRPS1 mut (Fig. 4B). Besides, we found that the protein expression of TRPS1 was decreased by miR-126-5p up-regulation and increased by miR-126-5p down-regulation in CHON-001 cells (all $P < 0.001$, Fig. 4C). The mRNA expression of TRPS1 was declined in the PTOA group compared to the control group ($P < 0.001$, Fig. 4D). TRPS1 was inversely correlated with miR-126-5p ($P = 0.0037$, $R^2 = 0.336$, Fig. 4E) and positively correlated with KCNQ1OT1 ($P < 0.0001$, $R^2 = 0.6136$, Fig. 4F) in cartilage tissues of PTOA patients.

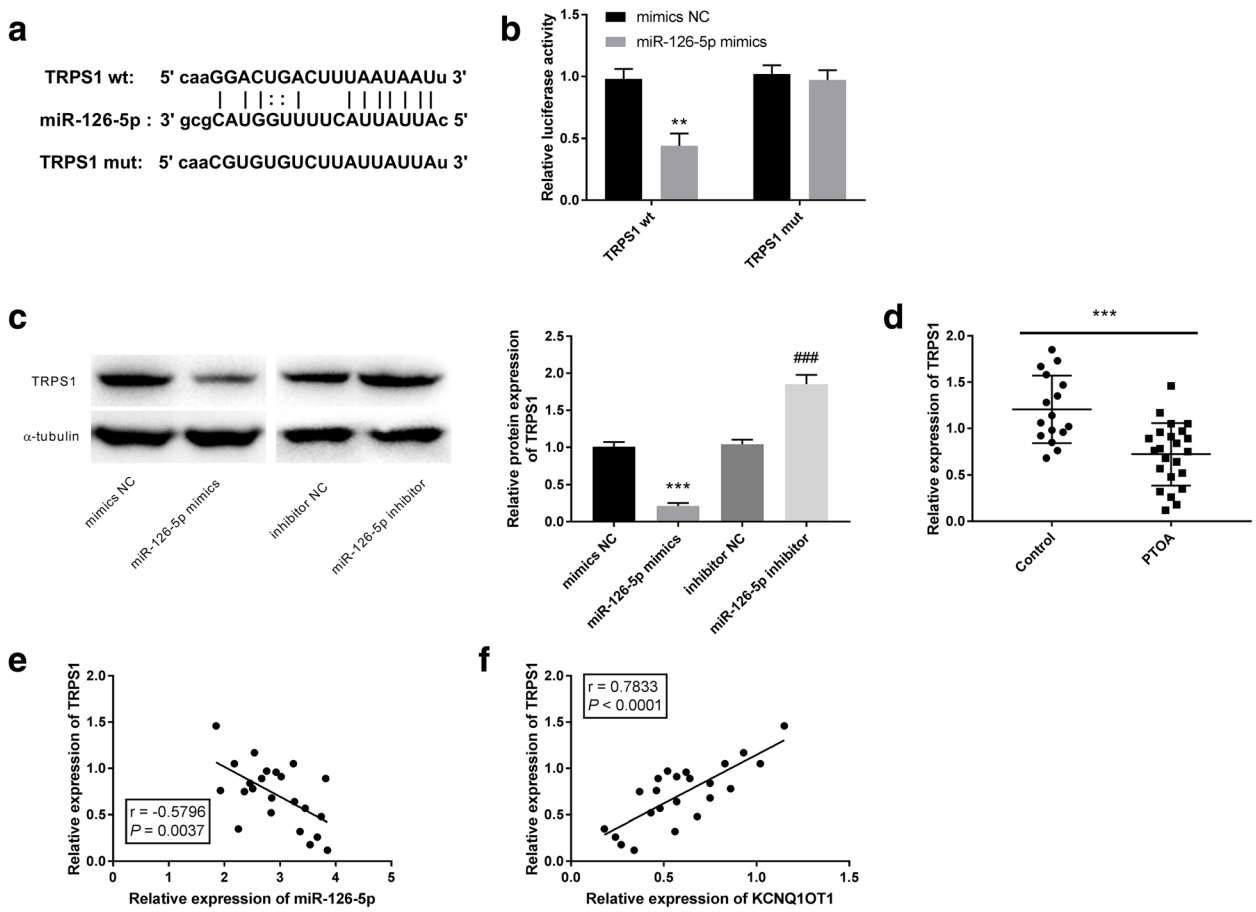

Fig. 4 TRPS1 was targeted by miR-126-5p. **a** The binding sequences between TRPS1 and miR-126-5p were predicted by starbase. **b** The interaction between TRPS1 and miR-126-5p in CHON-001 cells was verified by dual-luciferase reporter (DLR) assay. $^{**}P < 0.01$, vs. mimics NC. **c** Relative protein expression of TRPS1 was detected by western blot in CHON-001 cells. $^{***}P < 0.001$, vs. mimics NC. $^{\#\#\#}P < 0.001$, vs. inhibitor NC. **d** Relative expression of TRPS1 was detected by qRT-PCR in cartilage tissues of PTOA patients and normal cartilage tissues. $^{***}P < 0.001$, vs. Control. **e** The correlation between TRPS1 and miR-126-5p was analyzed by Pearson's correlation analysis in cartilage tissues of PTOA patients. $P = 0.0037$. $R^2 = 0.336$. **f** The correlation between KCNQ1OT1 and TRPS1 was analyzed by Pearson's correlation analysis in cartilage tissues of PTOA patients. $P < 0.0001$. $R^2 = 0.6136$

LncRNA KCNQ1OT1 enhances the viability and migration but suppresses degradation of CHON-001 cells via sponging miR-126-5p to target TRPS1

Finally, we explored the interrelation among KCNQ1OT1, miR-126-5p and TRPS1 in CHON-001 cells. Firstly, TRPS1 knockdown was achieved by sh-TRPS1 transfection. As expected, the mRNA expression of TRPS1 was remarkably reduced by sh-TRPS1 transfection in CHON-001 cells ($P < 0.01$, Fig. 5A). Then, the regulatory relation of KCNQ1OT1, miR-126-5p and TRPS1 was tested. We found that TRPS1 expression was elevated by pcDNA3.1-KCNQ1OT1, and the elevation effect of pcDNA3.1-KCNQ1OT1 on TRPS1 expression was reversed by miR-126-5p mimics in CHON-001 cells (all $P < 0.001$, Fig. 5B). Next, rescue assays were implemented. It was proved that KCNQ1OT1 overexpression strengthened the viability and migratory ability of CHON-001 cells (all $P < 0.001$, Fig. 5C-D), and these enhanced effects were partially attenuated by miR-126-5p overexpression or TRPS1 knockdown in CHON-001 cells (all $P < 0.01$, Fig. 5C-D). Additionally, KCNQ1OT1 overexpression elevated the protein expression of collagen II but reduced the protein expression of MMP13 in CHON-001 cells (all $P < 0.001$, Fig. 5E), which was reversed by miR-126-5p overexpression or TRPS1 knockdown (all $P < 0.01$, Fig. 5E).

Discussion

OA is the most common joint disease with high morbidity in the middle-aged and elderly population [25, 26]. Numerous lncRNAs have been confirmed to be down-regulated in OA [27–29], such as lncRNA ATB [27], lncRNA PACER [28], and lncRNA ANCR [29]. In line with the previous studies, a decreased expression of

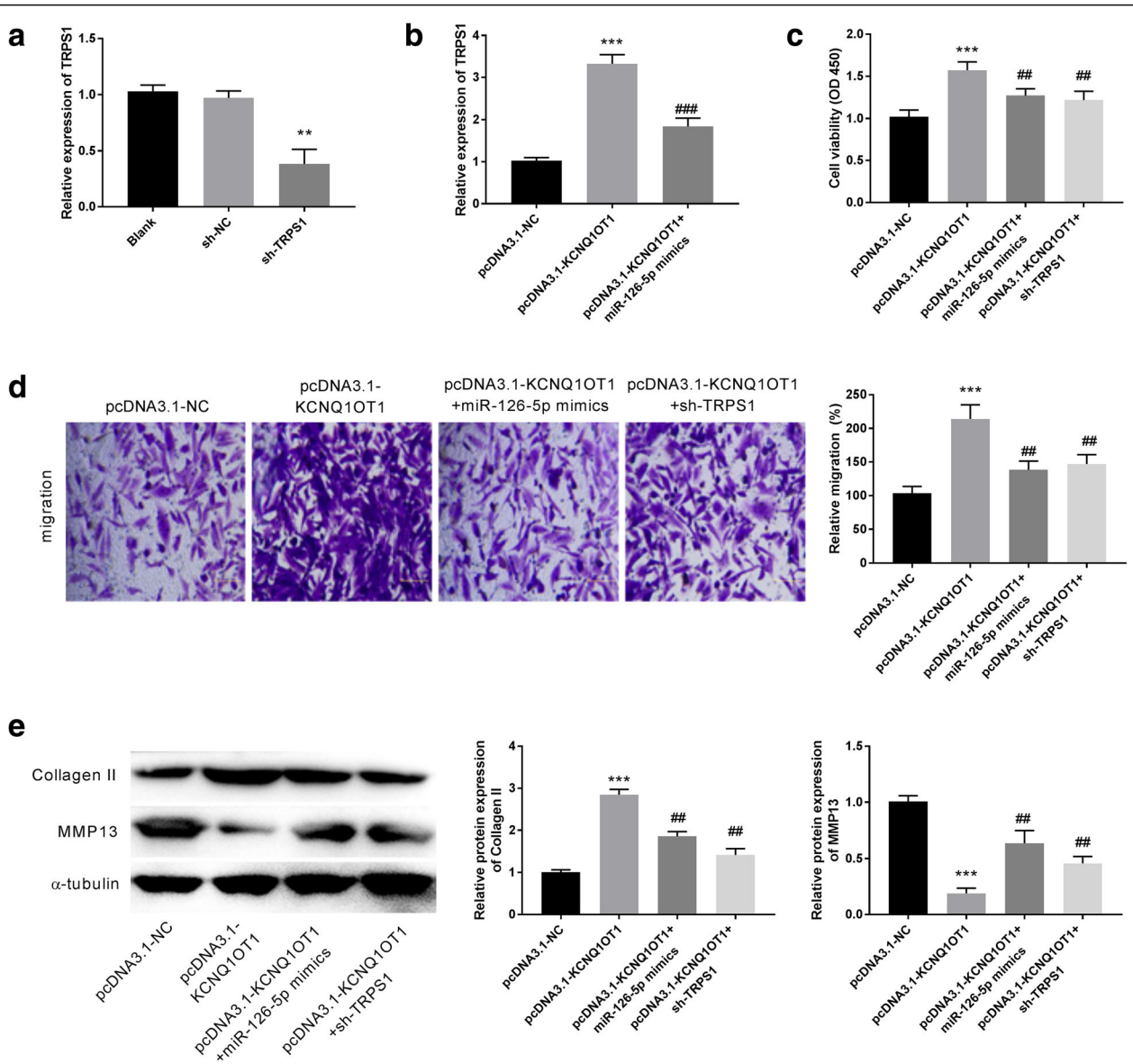

Fig. 5 LncRNA KCNQ1OT1 enhances the cell viability and migration but suppresses ECM degradation via sponging miR-126-5p to target TRPS1 in CHON-001 cells. **a** Relative expression of TRPS1 in CHON-001 cells was detected by qRT-PCR after transfection of sh-NC and sh-TRPS1. $^{**}P < 0.01$, vs. sh-NC. **b** Relative expression of TRPS1 was determined by qRT-PCR in CHON-001 cells. $^{***}P < 0.001$, vs. pcDNA3.1-NC. $^{###}P < 0.001$, vs. pcDNA3.1-KCNQ1OT1. **c** The cell viability was determined by MTT assay in CHON-001 cells. $^{***}P < 0.001$, vs. pcDNA3.1-NC. $^{##}P < 0.01$, vs. pcDNA3.1-KCNQ1OT1. **d** Relative migration was assessed by transwell assay in CHON-001 cells. $^{***}P < 0.001$, vs. pcDNA3.1-NC. $^{##}P < 0.01$, vs. pcDNA3.1-KCNQ1OT1. **e** Relative protein expression of collagen II and MMP13 was determined by western blot in CHON-001 cells. $^{***}P < 0.001$, vs. pcDNA3.1-NC. $^{##}P < 0.01$, vs. pcDNA3.1-KCNQ1OT1

lncRNA KCNQ1OT1 was also observed in cartilage tissues of PTOA patients compared to that of subjects without PTOA. The results implied that lncRNA KCNQ1OT1 may participate in the progression of OA. Given that loss of chondrocytes is the main cause of OA [30], numerous reports have focused on the impacts of lncRNAs on chondrocytes [8, 9, 31]. For instance, knockdown of lncRNA-p21 strengths cell viability and repress apoptosis in OA chondrocytes [31]. LncRNA MALAT1 promotes cell proliferation and inhibit ECM degradation in IL-1β-induced chondrocytes [8]. Silencing of lncRNA MFI2-AS1 elevates chondrocytes viability but represses ECM degradation [9]. In this study, we discovered that KCNQ1OT1 overexpression promoted the viability and migration as well as inhibition of ECM degradation in CHON-001 cells. Similarly, a recent study conducted by Gu et al. has indicated that KCNQ1OT1 facilitates the proliferation and suppress apoptosis of

articular chondrocytes, thus accelerating fracture healing [12]. Therefore, we speculated that KCNQ1OT1 may be a favourable factor to prevent the development of OA due to its important role in promoting the viability and migration of chondrocytes.

Emerging evidence has shown that miR-162 is implicated in OA [32]. Yu et al. have indicated that miR-126 is highly expressed in OA tissues, whereas decreased miR-126 strengthens chondrocyte viability and migration [18]. Interestingly, Ai et al. also found an increased miR-126 in CHON-001 cells, and the cell proliferation can be promoted by suppression of miR-126 [19]. Similarly, we revealed that miR-126-5p was up-regulated in cartilage tissues of PTOA patients, suggesting that miR-126-5p may be involved in the development of OA. Then, our functional experiments on miR-126-5p demonstrated that inhibition of miR-126-5p enhanced the viability and migratory ability of CHON-001 cells, and repressed ECM degradation in CHON-001 cells. Based on these results, we speculated that miR-126-5p may be a pathogenic miRNA existed in OA progression. On the other hand, miR-126-5p is revealed to act as the downstream targets of varied lncRNAs, such as lncRNA CRNDE [33], lncRNA HOTAIR [34], and lncRNA TMPO-AS1 [35]. Here, miR-126-5p was also identified as the target of and negatively regulated by lncRNA KCNQ1OT1. Therefore, we speculated that KCNQ1OT1 may promote the viability and migration of CHON-001 cells by targeting miR-126-5p. The rescue experiments confirmed that miR-126-5p mimics reversed the promoting effects of KCNQ1OT1 on cell viability and migration as well as the suppressive effect of KCNQ1OT1 on ECM degradation in CHON-001 cells. These results verified our speculation.

Numerous studies have uncovered the vital role of TRPS1 in cellular processes of chondrocytes [24, 36]. Gai et al. have stated that TRPS1 can regulate the proliferation and apoptosis of chondrocytes [36]. Suemoto et al. have unveiled that overexpression of TRPS1 facilitates chondrocytes proliferation by reduction the level of Stat3 [24]. In the present study, we found that TRPS1 expression was reduced in cartilage tissues, suggesting that TRPS1 may exhibited an anti-osteoarthritis OA progression. It is noticed that TRPS1, an atypical member of the GATA transcriptional factor family, is initially identified as a transcriptional repressor [37]. Indeed, it was previously uncovered to be targeted by varied miRNAs, including miR-26b-5p [38], and miR-221 [39]. Additionally, convincing evidence has demonstrated that complex interactions between miRNAs and their target genes are essential in regulating of homeostatic pathways in OA [40]. Inspired by this perception, bioinformatics analysis was implemented to predict target genes of miR-126-5p. We found that TRPS1 was a direct target gene of miR-126-5p. Meanwhile, we observed the negative correlation between TRPS1 and miR-126-5p in cartilage tissues of PTOA patients. Taken together, we speculated that decreased miR-126-5p enhanced the viability and migratory ability as well as repressing ECM degradation by targeting TRPS1 in CHON-001 cells. More importantly, we discovered that TRPS1 was positively correlated with KCNQ1OT1 in cartilage tissues of PTOA patients. The promoting effects of KCNQ1OT1 on cell viability and migration as well as the inhibitory effect of KCNQ1OT1 on ECM degradation were reversed by TRPS1 knockdown in CHON-001 cells. Therefore, we believed that lncRNA KCNQ1OT1 could repress OA progression via mediating miR-126-5p/TRPS1 axis in vitro. In addition, there may be some other important genes or pathways related to this axis in several types of human diseases, such as TRPS1-SOX9 in hereditary hypertrichoses or chondrocytes differentiation [41], KCNQ1OT1-PI3K/AKT pathway in cardiomyocytes injury [42] or colorectal cancer [43], miR-126-5p-PI3K/AKT pathway in ovarian cancer [44], cervical cancer [45], and gastric cancer [46]. We speculated that these important genes or pathways may be also involved in regulation of OA pathogenesis via interaction of KCNQ1OT1/miR-126-5p/TRPS1 axis. This is a research direction in our future studies.

Furthermore, there are also some other limitations in this study: i) lncRNA KCNQ1OT1 may be related with paternal allele, and the differences on KCNQ1OT1 expression in female and male OA patients are needed to be studied; ii), CHON-001 cells combined with cytokine stimulation and osmotic pressure to establish a vitro model for OA or use of the isolated chondrocytes from OA patients may be more rigorous and significant; iii) this study is focused on a cellular level, and in vivo experiments should be performed.

Conclusions

In summary, lncRNA KCNQ1OT1 was decreased in the PTOA group compared to the control group. LncRNA KCNQ1OT1 acted as a sponge of miR-126-5p, and TRPS1 was the downstream target of miR-126-5p. Overall, lncRNA KCNQ1OT1 repressed OA progression via binding to miR-126-5p and indirectly regulating TRPS1 in vitro. Hence, our study provided insights into the development of a novel target for treating OA.

Abbreviations

OA: Osteoarthritis; LncRNAs: Long non-coding RNA; QRT-PCR: Quantitative real-time polymerase chain reaction; DLR: Dual-luciferase reporter; ECM: Extracellular matrix; KCNQ1OT1: LncRNA KCNQ1 overlapping transcript 1; MiRNAs: microRNAs; TRPS1: Tricho-rhino-phalangeal syndrome type I; PVDF: Polyvinylidene difluoride; TBST: Tris buffered saline Tween

Acknowledgements

Not applicable.

Authors' contributions
All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding
Not applicable.

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
The current study was approved by the ethics committees of Chifeng Municipal Hospital, and all participants had signed written informed consents.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 14 January 2021 Accepted: 17 May 2021
Published online: 09 June 2021

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