LncRNA CRNED Hinders The Progression of Osteoarthritis By Epigenetic Regulation of DACT1

Ziqi Zhang (✉ zhang.ziqi@xjtu.edu.cn)

The Second Affiliated Hospital of Xi'an Jiaotong University <https://orcid.org/0000-0001-5467-499X>

Pei Yang

The Second Affiliated Hospital of Xi'an Jiaotong University

Chunsheng Wang

The Second Affiliated Hospital of Xi'an Jiaotong University

Run Tian

The Second Affiliated Hospital of Xi'an Jiaotong University

Research

Keywords: lncRNA CRNED, DACT1, H3K27 acetylation, osteoarthritis, chondrocyte-like ATDC5 cells

Posted Date: September 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-636574/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Osteoarthritis (OA) is mainly characterized by articular cartilage degeneration, synovial fibrosis, and inflammation. LncRNA CRNED (colorectal neoplasia differentially expressed) has been reported to be down-regulated in age-related OA, but its role in injury-induced OA needs to be further explored. In this study, an OA rat model was established by using anterior cruciate ligament transection, and the adenovirus-mediated CRNED overexpression (Ad-CRNED) or DACT1 (dapper antagonist of catenin-1) interference (sh-DACT1) vectors were injected into the rat model via tail vein. Besides, chondrocyte-like ATDC5 cells were treated with IL-1 β (10 ng/mL) to simulate OA conditions *in vitro*. We found that overexpression of CRNED alleviated cartilage damage and synovitis in OA rats, and suppressed IL-1 β -induced apoptosis, inflammation, and extracellular matrix (ECM) degradation in chondrocyte-like ATDC5 cells, while silencing DACT1 effectively antagonized the protective effect of CRNED both *in vivo* and *in vitro*. Mechanism studies revealed that DACT1 could act as a downstream target of CRNED. By recruiting p300, CRNED promoted the enrichment of H3K27ac in the DACT1 promoter, thus promoting DACT1 transcription. In addition, CRNED hindered the activation of the Wnt/ β -catenin pathway in IL-1 β -stimulated cells by inducing DACT1 expression. In conclusion, CRNED promoted DACT1 expression through epigenetic modification and restrained the activation of Wnt/ β -catenin signaling to impede the progression of OA.

Introduction

Osteoarthritis (OA) is a degenerative disease involving joints, and usually characterized by articular cartilage degeneration, synovial fibrosis and inflammation, subchondral bone sclerosis, and osteophyte formation [1]. In the world, among people older than 60, approximately 10% of men and 18% of women suffer from this disease [2]. The incidence of OA is high, and currently there are no effective drugs to prevent or reverse the disease progression [3]. Joint replacement is effective in the treatment of end-stage disease, but its prognosis is poor [4]. This prompts us to conduct a more in-depth study on the pathogenesis of OA, so as to provide new ideas for the early prevention of OA.

With the continuous advancement of high-throughput technologies such as transcriptome and whole-genome sequencing, people have gradually realized that non-coding RNAs account for the vast majority (98%) of the human genome, and protein-coding RNAs account for only a small portion (2%) [5]. Long non-coding RNAs (lncRNAs) are RNA molecules with limited protein coding ability due to the lack of open reading frames, and their length is greater than 200 nucleotides [6]. Recently, lncRNAs as novel non-coding RNAs have received widespread attention. Growing evidence indicates that some lncRNAs are involved in many biological and pathological regulation processes through epigenetic regulation (including acetylation, methylation and ubiquitination) [7–9].

LncRNA CRNDE (colorectal neoplasia differentially expressed) has been reported to play a carcinogenic effect in various human cancers, including colorectal cancer[10], liver cancer[11], osteosarcoma[12], and cervical cancer[13]. The mechanisms by which CRNED works are multiple, and a previous research

demonstrated that CRNED can promote the progression of colorectal cancer through epigenetic silencing gene expression [14]. In addition, CRNDE has been identified as a key gene in age-related OA, with decreased expression in OA [15]. However, the role of CRNDE in injury-related OA and the mechanisms involved need to be further studied.

It has been reported that the classic Wnt/ β -catenin signaling pathway is abnormally activated in OA, and effective inhibitors of the Wnt pathway can alleviate the severity of OA and reduce cartilage degeneration and synovitis *in vivo* [16]. The Dapper Antagonist of Catenin-1 (DACT1) gene is located at 14q22.3 and has been reported as an effective inhibitor of the Wnt signaling, playing an important role in the progression of many human cancers [17–19]. Moreover, SIRT1 regulates DACT1 expression through deacetylation [20]. In addition, a whole genome chip sequencing data showed that DACT1 was down-regulated in human OA knee tissues [21], suggesting its possible involvement in the occurrence of OA.

In this study, we demonstrated that CRNED was downregulated in the OA rat model, and its high expression impeded the development of OA. CRNED bound to DACT1, and knockdown of DACT1 functionally antagonized the protective effect of CRNED on cartilage injury. In addition, we found that CRNED might regulate DACT1 transcription through epigenetic modification. Our data may provide evidence for CRNED as a potential therapeutic target for OA.

Materials And Methods

Model Establishment

Healthy specific pathogen-free Sprague-Dawley (SD) rats (weighing 200 ± 20 g) were purchased from Vital River Laboratory Animal Technology (Beijing, China). Rats were fed in a clean animal room (21 ± 3 °C; relative humidity, $50 \pm 10\%$) with free access to food and drink under a 12 h dark/light cycle. Rats were randomly assigned into two main groups: (1) OA group: rats underwent anterior cruciate ligament transection surgery to destabilize the joints and induce OA. (2) Sham group: a similar incision was made in the right joint capsule of the rat. On the next day, adenovirus-mediated CRNED overexpression vector (Ad-CRNED) or DACT1 interference vector (sh-DACT1) were injected into the OA rat model via tail vein. The volume of adenovirus fluid injected at one time was 0.05-0.1 mL/10 g per body weight, and the dosage of adenovirus solution was 2×10^9 PFU per rat.

All animal experiments were approved by the Second Affiliated Hospital of Xi'an Jiaotong University Animal Ethics Committee and were performed in accordance with the Committee's guidelines.

Cell Differentiation and Culture

ATDC5 cell line was purchased from Riken BioResource Center (Tsukuba, Japan). Undifferentiated ATDC5 cells were cultured in DMEM/F12 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 5% fetal bovine serum (Gibco, Rockville, MD) and 1% P/S (penicillin/streptomycin) at 37 °C in 5% CO₂. When the cell confluence reaches about 75%, 1% insulin-transferrin-selenite (ITS) is added to the

medium, and then the medium was changed every two days to induce the cells to differentiate into chondrocyte-like cells. Cells were used for the subsequent experiments after 2 weeks of differentiation in culture.

Cell Transfection

Adenovirus-mediated CRNED, DACT1 and p300 overexpression vectors were constructed using full-length sequences of CRNED, DACT1 and p300. The adenovirus vectors containing small hairpin RNA (shRNA) sequence targeting CRNED (sh-CRNED), DACT1 (sh-DACT1), p300 (sh-p300) or negative control vector (sh-NC) were amplified and cloned by GeneChem (Shanghai, China). All transfection was performed using Lipofectamine® 3000 reagent (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Proliferation

Cells were seeded into 96-well plates and incubated at 37 °C under 5% CO₂ for 24 h. After treatment, 10 µL of MTT reagent was added into each well, and then cells were incubated at 37°C for 4-6 h. After that, the supernatant was discarded and 100 µL of DMSO was added into each well. The absorbance of each well was measured at a wavelength of 450 nm after the crystals were sufficiently dissolved.

Cell Apoptosis

Apoptosis analysis was carried out by using Annexin V-FITC/propidium iodide (PI) apoptosis detection kit (Multisciences, Shanghai, China). In brief, the treated cells were washed with pre-cooled PBS and cell suspension was prepared. Next, 100 µL of cell suspension was incubated with 5 µL of Annexin V-FITC and 5 µL of PI in the dark for 20 min. Cell apoptosis was detected by using BD Accuri flow cytometer (BD Biosciences).

ELISA

The concentrations of IL-6, IL-8, and TNF-α in cell supernatant were measured using ELISA kits (R&D Systems, USA) according to the manufacturer's instructions. The absorbance of each well at the wavelength of 450 nm were measured. The experiment was repeated three times to obtain the mean value.

Western Blotting

Total protein extracted from cells and rat cartilage tissues was lysed with RIPA lysis buffer. CellLytic™ NuCLEAR™ Extraction Kit (Sigma, St. Louis, MO, USA) was used to extract nuclear proteins. Protein concentrations were determined by using the BCA Assay Kit (Beyotime, Shanghai, China). Equal amounts of protein were separated on 10% SDS/PAGE gels and then transferred onto PVDF membranes (Millipore, Billerica, MA, USA). After blocking in 5% skim milk at room temperature for 2 h, membranes were incubated at 4 °C overnight with primary antibodies including anti-ADAMTS-5 (ab41037, 1:250, Abcam, Cambridge, UK), anti-MMP13 (ab51072, 1:1000, Abcam), anti-Collagen 2 (ab188570, 1:1000, Abcam),

anti-Aggrecan (ab3778, 1:1000, Abcam), anti- β -catenin (ab68183, 1:500, Abcam), anti-DACT1 (ab42547, 1:1000, Abcam), anti-c-Myc (ab32072, 1:1000, Abcam) and anti-Cyclin D1 (ab16663, 1:200, Abcam). Then, membranes were incubated with HRP-conjugated specific secondary antibodies for 2 h at room temperature. Protein band signals were measured using enhanced chemiluminescence reagents (Beyotime). Image J software was used to analyze the gray level of bands.

RT-qPCR

Total RNA was extracted from cells and rat cartilage tissues using TRIzol kit (Invitrogen, Carlsbad, CA, USA). 1 μ g of RNA was reverse transcribed to cDNA using a Reverse Transcription Kit (Takara, Dalian, China). Synthesized cDNAs were used for RT-qPCR by using SYBR Premix Ex Taq Kit (Takara). The thermal cycling conditions were as follows: 3 minutes at 95 °C, 35 cycles for 15 seconds at 95 °C, 30 seconds at 60 °C, and 15 seconds at 72 °C. The relative mRNA levels were normalized to GAPDH and calculated by using $2^{-\Delta\Delta CT}$ method. DACT1: 5'-GGA AGA GGA CAG GCT TGG AAA C-3' (S) and 5'-GTC CCA TTG TTC AGA GAA GGT ATC-3' (R). CRNED: 5'-TGA AGG AAG GAA GTG GTG CA-3' (S) and 5'-TCC AGT GGC ATC CTA CAA GA-3' (R).

Chromatin Immunoprecipitation (CHIP) Assay

ChIP assay was carried out using the EZ ChIP™ Chromatin Immunoprecipitation Kit (Millipore, Burlington, MA) in accordance with the manufacturer's protocol. In brief, cells were incubated with formaldehyde for 10 min to produce DNA-protein crosslinks. Next, the cell lysates were sonicated into 200-1000 bp chromatin fragments and immunoprecipitated with anti-H3K27ac (ab4729, Abcam) and anti-p300 (ab2832, Abcam).

RNA Immunoprecipitation (RIP) Assay

RIP assay was performed by using Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore). In brief, the treated cells were washed by ice-cold PBS and lysed in a radioimmuno-precipitation buffer for 30 minutes at 4 °C. Next, the whole cell lysate was incubated with A/G sepharose beads coupled with anti-DACT1 (Abcam) or normal human IgG (Millipore) antibodies. The precipitated RNA was analyzed by qPCR.

RNA pull-down Assay

The transcribed RNA was biotin-labeled with Biotin RNA Labeling Mix (Ambio Life), treated with RNase-free DNase I (Roche, Indianapolis, IN, USA), and purified with an RNeasy Mini Kit (Qiagen, Valencia, CA). 1 mg of whole-cell lysates were incubated with 3 μ g of purified biotinylated transcripts at room temperature for 1 h. And then the complex was isolated with streptavidin agarose beads (Invitrogen, San Diego, CA, USA). The eluted proteins were measured by Western blot analysis.

Hematoxylin and Eosin (H&E) and Safranin O-fast Ereen Staining

The knee joints were fixed with formalin, decalcified with 0.5 M EDTA at pH 8.0 for 4 weeks, and dehydrated and embedded in paraffin. The tissue sections were cut into 4-6 μm thickness along the sagittal direction. Next, H&E and Safranin O-Fast Green staining was performed. At least five visual fields of each picture were selected to assess the severity of OA by using the Osteoarthritis Research Society International (OARSI) scoring system [22].

Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick-end Labeling (TUNEL) Assay

Paraffin sections of knee joint cartilage tissues were stained using TUNEL (Roche, Basel, Switzerland) method in accordance with the manufacturer's protocol. After staining, normal cells showed purple-blue, and apoptotic cells showed yellow-brown. TUNEL-positive cells were evaluated ($\times 100$) in six randomly-selected fields, and the average number of apoptotic cells was calculated.

Statistical Analysis

All data in this study were analyzed using SPSS 22.0 software (IBM Corp., Armonk, NY). Data were presented as mean $\pm$ standard error of mean (mean $\pm$ SEM). Differences between two groups were analyzed by using Student's *t* test. And comparisons among multiple groups were measured by using one-way analysis of variance (ANOVA). The level of significance was set at $*P < 0.05$.

Results

Overexpression of CRNED alleviated cartilage injury in an OA rat model

In order to explore the role of CRNED in OA, we used anterior cruciate ligament transection to establish an OA rat model. Our results showed that CRNED expression was significantly reduced in OA rats compared with that in the sham group, and the injection of Ad-CRNED observably increased the expression of CRNED (Fig. 1a). Next, optical microscopy was used to observe the pathological changes of knee cartilage in each group. As shown in Figure 1B, in the sham group, the cartilage surface was smooth, and chondrocytes were orderly arranged with no cartilage hyperplasia, cartilage sclerosis and synovial hyperplasia, and no significant inflammatory cells and blood vessels were observed in the synovial tissues. OA rats had severe articular cartilage wear, synovial hyperplasia, inflammatory cell infiltration and abnormal proliferation of chondrocytes, which was improved by overexpressing CRNED (Fig. 1b). Besides, the enforced expression of CRNED alleviated OA-induced synovial hyperplasia, and increased inflammatory cell infiltration and small vessels in synovial tissues (Fig. 1b). Moreover, OA group showed more Safranin O loss and cartilage fibrosis and a higher OARSI score than the sham group, which was also alleviated by the high expression of CRNED (Fig. 1c, d). In addition, Immunostaining analysis indicated that CRNED overexpression notably reversed the downregulation of Collagen 2 and Aggrecan expression (Fig. 1e). We also found that high expression of CRNED rescued OA-induced apoptosis of chondrocytes in the knee joint (Fig. 1f).

Overexpression of CRNED promoted cell proliferation and suppressed apoptosis, inflammation and ECM degradation in IL-1 β -induced chondrocyte-like ATDC5 cells

To further explore the role of CRNED in OA, IL-1 β was used to stimulate chondrocyte-like ATDC5 cells to simulate OA condition. Meanwhile, CRNED was overexpressed in IL-1 β -induced cells. Firstly, the CRNED expression was measured, and it was downregulated after IL-1 β treatment, but increased after transfection with Ad-CRNED (Fig. 2a). Besides, IL-1 β induction lead to reduced cell proliferation, and increased apoptosis and inflammatory factor (including IL-6, IL-8 and TNF- α) secretion, which was partially abolished by CRNED overexpression (Fig. 2b-d). Moreover, the expression of ECM degradation-related biomarkers was evaluated, and higher expression of MMP13 and ADAMTS-5, and lower protein levels of Collagen 2 and Aggrecan were observed in IL-1 β treatment group than in control group (Fig. 2e). However, overexpression of CRNED reversed the promotion effect of IL-1 β on ECM degradation (Fig. 2e).

CRNED targeted DACT1 and modulated its expression

It is reported that DACT1 is down-regulated in OA knee tissues, and its low expression may be related to the progression of OA [21]. So we determined DACT1 expression in OA *in vivo* and *in vitro* models, and the data displayed that the mRNA and protein levels of DACT1 were decreased both in OA rat tissues and IL-1 β -treated chondrocyte-like ATDC5 cells compared with that in normal cartilaginous tissues and untreated cells (Fig. 3a, b). Next, in order to explore whether DACT1 was a downstream target of CRNED, RIP and RNA pull down assays were performed. The results in RIP assay showed the significant enrichment of CRNED using DACT1 antibody compared with IgG antibody (Fig. 3c). And RNA pull-down assay showed a prominent enrichment of DACT1 protein in CRNED-protein complexes (Fig. 3d). Furthermore, we also found that the mRNA and protein levels of DACT1 was upregulated after overexpressing CRNED, while downregulated after silencing CRNED (Fig. 3e, f).

DACT1 inhibition abolished the protective effects of CRNED on IL-1 β -stimulated chondrocyte-like ATDC5 cells

In order to study whether CRNED modulated cell functions by regulating DACT1 expression, Ad-CRNED was transfected into IL-1 β -stimulated cells alone, or together with sh-DACT1. The results indicated that CRNED overexpression upregulated the mRNA and protein levels of DACT1 in IL-1 β -treated cells, which was observably reduced by transfection with sh-DACT1 (Fig. 4a, b). Moreover, silencing DACT1 markedly hindered the reversal effect of CRNED overexpression on decreased cell proliferation, and increased apoptosis and inflammatory mediator (including IL-6, IL-8 and TNF- α) release caused by IL-1 β (Fig. 4c-e). In addition, the knockdown of DACT1 also abolished the inhibitory action of CRNED on IL-1 β -induced ECM degradation by upregulating MMP13 and ADAMTS-5 expression and downregulating the protein levels of Collagen 2 and Aggrecan (Fig. 4f).

Overexpression of CRNED promoted DACT1 expression by regulating H3K27 acetylation

To deeply investigate the regulatory relationship between CRNED and DACT1, we explored the possible regulatory mechanisms by using online databases of UCSC and ENCODE (Fig. 5a). We found that H3K27ac was significantly enriched in the promoter region of DACT1, and CRNED was mainly located in nucleus (Fig. 5b). Therefore, we speculated that CRNED might regulate the DACT1 expression through histone modification at the transcriptional level. To test our hypothesis, CHIP assay was performed in chondrocyte-like ATDC5 cells. The data showed that DACT1 promoter had a significant enrichment in the H3K27ac antibody-precipitated complex compared to the IgG (Fig. 5c). Besides, a notably reduced enrichment of H3K27ac was observed in IL-1 β -treated cells compared to normal cells (Fig. 5d). We also evaluated the effect of CRNED on the enrichment of H3K27ac in the DACT1 promoter region, and the results displayed that overexpression of CRNED promoted the enrichment of H3K27ac, while the silence of CRNED hindered the H3K27ac enrichment (Fig. 5e, f). In addition, a histone acetyltransferase inhibitor (C646) was used to treat chondrocyte-like ATDC5 cells, and the data indicated a remarkable reduction in DACT1 mRNA and protein levels after treatment (Fig. 5g).

CRNED regulated H3K27 acetylation at the promoter of DACT1 by binding to p300

Since C646 is an effective inhibitor of p300, the role of p300 in the regulatory effect of CRNED on DACT1 expression was investigated. We first detected whether the expression of DACT1 responded to changes in p300 expression levels, and the results revealed that the mRNA and protein levels of DACT1 was prominently upregulated after overexpressing p300, and downregulated after interference with p300 (Fig. 6a, b). The CHIP assay showed a prominent enrichment of DACT1 promoter in the p300 antibody-precipitated complex compared to the IgG (Fig. 6c). Moreover, RIP assay was carried out to measure the interaction between CRNED and p300. As shown in Fig. 6d, a significant amount of CRNED was detected using p300 antibody compared to IgG antibody, and treatment with IL-1 β attenuated this interaction between them (Fig. 6d, e). Besides, we found that p300 could also regulate the enrichment of H3K27ac in the DACT1 promoter region. And overexpressing p300 increased the enrichment of H3K27ac, while silencing p300 played the opposite role (Fig. 6f, g). In addition, a markedly increased enrichment of p300 in the DACT1 promoter region was observed in response to overexpression of CRNED, while interference with CRNED showed the opposite effect (Fig. 6h, i). However, the mRNA and protein levels of p300 were not altered by either high or low expression of CRNED (Fig. 6j, k). Our results suggested that CRNED might function by recruiting CBP to target downstream genes.

CRNED restrained IL-1 β -induced activation of Wnt/ β -catenin signaling by regulating DACT1 in chondrocyte-like ATDC5 cells

DACT1 is reported to be an effective inhibitor of the Wnt signaling pathway [23]. We overexpressed and interfered DACT1 in chondrocyte-like ATDC5 cells, and measured Wnt signaling and its downstream gene expression. The protein levels of β -catenin in nucleus, and c-Myc and Cyclin D1 in the whole cells were downregulated in Ad-DACT1 transfection group, but increased in DACT1 interference group (Fig. 7a, b). To explore whether CRNED regulated Wnt signaling pathway activity through DACT1, Ad-CRNED was transfected into IL-1 β -stimulated cells alone, or together with sh-DACT1. IL-1 β treatment resulted in a

significant increase in the expression of β -catenin, c-Myc and Cyclin D1, which was rescued by overexpression of CRNED (Fig. 7c, d). Besides, interference with DACT1 abrogated the effect of CRNED on Wnt/ β -catenin pathway activity (Fig. 7c, d). Next, we replaced the sh-DACT1 with HY-122816 (a specific activator of the Wnt/ β -catenin pathway). Similar to interference with DACT1, HY-122816 treatment could also hinder the decrease of β -catenin, c-Myc and Cyclin D1 expression caused by CRNED overexpression in IL-1 β -induced cells (Fig. 7e, f).

Overexpression of CRNED mitigated cartilage injury in an OA rat model by modulating DACT1

Ad-CRNED was administration alone or together with sh-DACT1 in the OA rat model to study the effect of DACT1 in OA progress. The data showed that DACT1 expression was downregulated in OA rats. And sh-DACT1 injection reversed CRNED-induced increase in DACT1 expression in OA rats (Fig. 8a). As shown in Fig. 8b-d, HE staining and Safranin O staining displayed that overexpression of CRNED alleviated OA-induced cartilage wear and fibrosis, synovial hyperplasia, loss of Safranin O, and increased OARSI score. Interference with DACT1 hindered the protective effect of CRNED on knee joint damage (Fig. 8b-d). In addition, silencing DACT1 could also reverse the inhibitory effect of CRNED overexpression on OA-induced apoptosis of chondrocytes in the knee joint (Fig. 8e).

Discussion

OA is induced by many factors, including obesity, mechanical injury, aging, heredity, etc. It usually occurs in the knee, spine and hip, and patients show swelling and pain in the joints and decreased ability to walk [24]. Healthy cartilage consists mainly of chondrocytes[25]. Chondrocytes can produce cartilage extracellular matrix rich in collagen (mainly type II collagen) and proteoglycans. Under pathological conditions, chondrocytes can also produce mechanisms such as metalloproteinases to degrade cartilage matrix [26]. In addition, after the occurrence of OA, chondrocyte functions including proliferation and secretion have undergone many changes [27].

It is reported that lncRNAs regulate various biological processes, including proliferation, metastasis, apoptosis, cell cycle transformation and cell structural integrity, through chromatin remodeling, adsorption of miRNAs and epigenetic modification [28, 29]. The potential importance of lncRNAs in OA has been revealed by Fu et al. [30]. CRNED is up-regulated in many solid and hematopoietic cancers, and its high expression contributes to malignant progression of cancers [31–33]. But in osteoblasts, CRNED appeared to be protective, and it promoted proliferation and differentiation of osteoblasts and could be used as a novel regulator of bone metabolism [34]. In addition, in age-induced OA, CRNDE is low-expressed and may serve as a key regulator for OA [15]. Our findings indicated that overexpression of CRNED alleviated knee cartilage damage and synovitis in the OA rat model. *In vitro*, the high expression of CRNED hindered IL-1 β -induced apoptosis, inflammation and ECM degradation in chondrocyte-like ATDC5 cells. Our data indicated the protective role of CRNED in OA.

It is generally believed that the Wnt/ β -catenin signaling pathway is a unique signaling pathway that regulates the occurrence and development of OA. Numerous studies have shown that Wnt/ β -catenin

signaling plays an important role in joint pathology by acting directly on bone, cartilage and synovial tissues [35, 36]. Consistent with previous reports, our results showed that the Wnt signaling was activated in IL-1 β -stimulated chondrocyte-like ATDC5 cells, and the expression of its downstream genes c-Myc and Cyclin D1 was also up-regulated. As we all know, DACT1 is an inhibitor of the Wnt pathway and can also regulate the expression of Wnt downstream genes [23]. We found that overexpression of DACT1 suppressed the activity of Wnt signaling in chondrocyte-like ATDC5 cells. Both interference with DACT1 and treatment with a Wnt-specific activator (HY-122816) effectively reversed the inactivation of the Wnt pathway caused by CRNED overexpression. DACT1 plays a dual role in cancers. It can not only act as a tumor suppressor in breast and liver cancers [23, 37], but also promote the malignant progression of colon and ovarian cancers [17, 38]. In addition, the sequencing data of Chou et al. indicated that DACT1 was lower expressed in OA knee tissues than in normal knee tissues [21]. In *in vivo* and *in vitro* OA models, DACT1 expression was significantly reduced. And silencing DACT1 partially abrogated the protective effects of CRNED on IL-1 β -induced apoptosis, inflammation, and ECM degradation in chondrocyte-like ATDC5 cells, demonstrating that DACT1 acted as a downstream target gene of CRNED function.

Increasing evidence shows that lncRNAs can activate or inhibit target gene expression through epigenetic modifications (including acetylation, methylation, ubiquitination, phosphorylation) [39]. The mechanism of lncRNAs is closely related to its cellular localization, and lncRNAs located in the nucleus are likely to regulate gene expression through transcriptional regulation and chromatin modification [40]. The results in this study displayed that CRNED was mainly expressed in the nucleus, suggesting that it might affect gene expression through epigenetic modifications. CRNED has been reported to promote EZH2-mediated methylation modification of target genes by binding to EZH2, thereby silencing gene expression [14]. We found that CRNED could bind to DACT1 and might modulate its expression by mediating histone acetylation. Acetylation is the most common histone acyl modification and a large number of studies have demonstrated the relationship between increased histone acetylation levels and transcriptional activation [41]. Histone acetylation modification regulated by lncRNA has been widely reported. IL-7-AS-V1 regulates H3K9ac and H3K14ac at several inflammatory gene promoters, and promotes gene expression by recruiting histone acetyltransferase p300 [42]. lncRNA AGAP2-AS1 increases MyD88 expression by promoting H3K27ac in the MyD88 promoter [43]. Moreover, it is reported that SIRT1 regulates DACT1 expression through deacetylation [20]. The bioinformatics analysis showed that H3K27ac enriched significantly in the promoter region of DACT1, and CRNED could regulate this histone modification, which was manifested as highly expressed CRNED promoted the enrichment of H3K27ac. In addition, IL-1 β stimulation restrained the enrichment of H3K27ac in DACT1 promoter in chondrocyte-like ATDC5 cells.

According to reports, many lncRNAs regulate gene expression by binding to various histone-modifying enzymes to affect cell functions [14]. For example, lncRNA Khps1 upregulates gene expression through recruitment of histone acetyltransferase p300 / CBP [44]. P300 is a global co-activator of various transcription factors with histone acetyltransferase (HAT) activity [45]. Besides, it is reported that RNA can directly bind to a unique region in the HAT domain of p300 to stimulate the HAT activity of p300 [46].

Our data revealed that CRNED modulated H3K27ac enrichment in the DACT1 promoter by recruiting p300 to the DACT1 promoter and binding to p300 to form a complex, which ultimately affected the expression of DACT1.

In conclusion, our study provided evidence that high expression of CRNED might hinder the progression of OA. And the mitigation effect of CRNED on knee articular cartilage injury *in vivo*, and chondrocyte damage *in vitro* might be achieved by epigenetic promotion of DACT1 transcription by binding to p300. CRNDE might be used as a promising target for OA diagnosis and treatment.

Declarations

Data availability statement

All data used during the current study are available from the corresponding author on reasonable request.

Ethics statement

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University. All methods were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH).

Author contributions

ZQZ: research design, manuscript preparation and data analysis; PY: experimental material preparation and rat feeding; CHW: completion of molecular biology experiments; RT: histology analysis.

Funding

Not applicable.

Acknowledgments

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Millerand M, Berenbaum F, Jacques C (2019) Danger signals and inflammaging in osteoarthritis. Clin Exp Rheumatol 37(Suppl 120):48–56
2. Glyn-Jones S, Palmer AJR, Agricola R, Price AJ, Vincent TL, Weinans H, Carr AJ (2015) Osteoarthritis The Lancet 386(9991):376–387

3. Zhang Z, Leong DJ, Xu L, He Z, Wang A, Navati M, Kim SJ, Hirsh DM, Hardin JA, Cobelli NJ, Friedman JM, Sun HB (2016) Curcumin slows osteoarthritis progression and relieves osteoarthritis-associated pain symptoms in a post-traumatic osteoarthritis mouse model. *Arthritis Res Ther* 18(1):128
4. Carr AJ, Robertsson O, Graves S, Price AJ, Arden NK, Judge A, Beard DJ (2012) Knee replacement. *Lancet* 379(9823):1331–1340
5. Sun L, Goff LA, Trapnell C, Alexander R, Lo KA, Hacisuleyman E, Sauvageau M, Tazon-Vega B, Kelley DR, Hendrickson DG (2013) Long noncoding RNAs regulate adipogenesis. *P Nal Acad Sci USA* 110(9):3387–3392
6. Quinn JJ, Chang HY (2016) Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet* 17(1):47
7. Xing Z, Lin A, Li C, Liang K, Wang S, Liu Y, Park PK, Qin L, Wei Y, Hawke DH (2014) lncRNA directs cooperative epigenetic regulation downstream of chemokine signals. *Cell* 159(5):1110–1125
8. Zhou J, Yang L, Zhong T, Mueller M, Men Y, Zhang N, Xie J, Giang K, Chung H, Sun X (2015) H19 lncRNA alters DNA methylation genome wide by regulating S-adenosylhomocysteine hydrolase. *Nat Commun* 6(1):1–13
9. Ni W, Zhang Y, Zhan Z, Ye F, Liang Y, Huang J, Chen K, Chen L, Ding Y (2017) A novel lncRNA uc. 134 represses hepatocellular carcinoma progression by inhibiting CUL4A-mediated ubiquitination of LAT51. *J Hematol Oncol* 10(1):91
10. Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, Liu YL, Cui BB (2017) The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/beta-catenin signaling. *Mol Cancer* 16(1):9
11. Chen Z, Yu C, Zhan L, Pan Y, Chen L, Sun C (2016) lncRNA CRNDE promotes hepatic carcinoma cell proliferation, migration and invasion by suppressing miR-384. *Am J Cancer Res* 6(10):2299
12. Li Z, Tang Y, Xing W, Dong W, Wang Z (2018) lncRNA, CRNDE promotes osteosarcoma cell proliferation, invasion and migration by regulating Notch1 signaling and epithelial-mesenchymal transition. *Exp Mol Pathol* 104(1):19–25
13. Bai X, Wang W, Zhao P, Wen J, Guo X, Shen T, Shen J, Yang X (2020) lncRNA CRNDE acts as an oncogene in cervical cancer through sponging miR-183 to regulate CCNB1 expression. *Carcinogenesis* 41(1):111–121
14. Ding J, Li J, Wang H, Tian Y, Xie M, He X, Ji H, Ma Z, Hui B, Wang K, Ji G (2017) Long noncoding RNA CRNDE promotes colorectal cancer cell proliferation via epigenetically silencing DUSP5/CDKN1A expression. *Cell Death Dis* 8(8):e2997
15. Hu P, Sun F, Ran J, Wu L (2019) Identify CRNDE and LINC00152 as the key lncRNAs in age-related degeneration of articular cartilage through comprehensive and integrative analysis. *PeerJ* 7:e7024
16. Lietman C, Wu B, Lechner S, Shinar A, Sehgal M, Rossomacha E, Datta P, Sharma A, Gandhi R, Kapoor M, Young PP (2018) Inhibition of Wnt/beta-catenin signaling ameliorates osteoarthritis in a murine model of experimental osteoarthritis. *JCI Insight* 3(3)

17. Li RN, Liu B, Li XM, Hou LS, Mu XL, Wang H, Linghu H (2017) DACT1 Overexpression in type I ovarian cancer inhibits malignant expansion and cis-platinum resistance by modulating canonical Wnt signalling and autophagy. *Sci Rep* 7(1):9285
18. Yang JH, Lin LK, Zhang S (2019) Effects of DACT1 methylation status on invasion and metastasis of nasopharyngeal carcinoma. *Biol Res* 52(1):31
19. Zhu K, Jiang B, Yang Y, Hu R, Liu Z (2017) DACT1 overexpression inhibits proliferation, enhances apoptosis, and increases daunorubicin chemosensitivity in KG-1alpha cells. *Tumour Biol* 39(10):1010428317711089
20. Zhou Y, Song T, Peng J, Zhou Z, Wei H, Zhou R, Jiang S, Peng J (2016) SIRT1 suppresses adipogenesis by activating Wnt/ β -catenin signaling in vivo and in vitro. *Oncotarget* 7(47):77707
21. Chou CH, Lee MT, Song IW, Lu LS, Shen HC, Lee CH, Wu JY, Chen YT, Kraus VB, Wu CC (2015) Insights into osteoarthritis progression revealed by analyses of both knee tibiofemoral compartments. *Osteoarthritis Cartilage* 23(4):571–580
22. Glasson SS, Chambers MG, Van Den Berg WB, Little CB (2010) The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage* 18(Suppl 3):S17–S23
23. Yin X, Xiang T, Li L, Su X, Shu X, Luo X, Huang J, Yuan Y, Peng W, Oberst M (2013) DACT1, an antagonist to Wnt/ β -catenin signaling, suppresses tumor cell growth and is frequently silenced in breast cancer. *Breast Cancer Res* 15(2):1–12
24. Bai Z, Guo XH, Tang C, Yue ST, Shi L, Qiang B (2018) Effects of Artesunate on the Expressions of Insulin-Like Growth Factor-1, Osteopontin and C-Telopeptides of Type II Collagen in a Rat Model of Osteoarthritis. *Pharmacology* 101(1–2):1–8
25. Berenbaum F (2013) Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis Cartilage* 21(1):16–21
26. Wang C, Yao Z, Zhang Y, Yang Y, Liu J, Shi Y, Zhang C (2020) Metformin Mitigates Cartilage Degradation by Activating AMPK/SIRT1-Mediated Autophagy in a Mouse Osteoarthritis Model. *Front Pharmacol* 11:1114
27. Charlier E, Deroyer C, Ciregia F, Malaise O, Neuville S, Plener Z, Malaise M, de Seny D (2019) Chondrocyte dedifferentiation and osteoarthritis (OA). *Biochem Pharmacol* 165:49–65
28. Xu Y, Ge Z, Zhang E, Zuo Q, Huang S, Yang N, Wu D, Zhang Y, Chen Y, Xu H, Huang H, Jiang Z, Sun L (2017) The lncRNA TUG1 modulates proliferation in trophoblast cells via epigenetic suppression of RND3. *Cell Death Dis* 8(10):e3104
29. Jiang SD, Lu J, Deng ZH, Li YS, Lei GH (2017) Long noncoding RNAs in osteoarthritis. *Joint Bone Spine* 84(5):553–556
30. Fu M, Huang G, Zhang Z, Liu J, Huang Z, Yu B, Meng F (2015) Expression profile of long noncoding RNAs in cartilage from knee osteoarthritis patients. *Osteoarthritis Cartilage* 23(3):423–432
31. Ma X, Zhang W, Zhao M, Li S, Jin W, Wang K (2020) Oncogenic role of lncRNA CRNDE in acute promyelocytic leukemia and NPM1-mutant acute myeloid leukemia. *Cell Death Discov* 6(1):1–13

32. Sun H, He L, Ma L, Lu T, Wei J, Xie K, Wang X (2017) LncRNA CRNDE promotes cell proliferation, invasion and migration by competitively binding miR-384 in papillary thyroid cancer. *Oncotarget* 8(66):110552
33. Xie S-C, Zhang J-Q, Jiang X-L, Hua Y-Y, Xie S-W, Qin Y-A, Yang Y-J (2020) LncRNA CRNDE facilitates epigenetic suppression of CELF2 and LATS2 to promote proliferation, migration and chemoresistance in hepatocellular carcinoma. *Cell Death Dis* 11(8):1–17
34. Mulati M, Kobayashi Y, Takahashi A, Numata H, Saito M, Hiraoka Y, Ochi H, Sato S, Ezura Y, Yuasa M, Hirai T, Yoshii T, Okawa A, Inose H (2020) The long noncoding RNA Crnde regulates osteoblast proliferation through the Wnt/beta-catenin signaling pathway in mice. *Bone* 130:115076
35. Wang Y, Fan X, Xing L, Tian F (2019) Wnt signaling: a promising target for osteoarthritis therapy. *Cell Commun Signal* 17(1):97
36. Zhou Y, Wang T, Hamilton JL, Chen D (2017) Wnt/beta-catenin Signaling in Osteoarthritis and in Other Forms of Arthritis. *Curr Rheumatol Rep* 19(9):53
37. Tuo H, Wang Y, Wang L, Yao B, Li Q, Wang C, Liu Z, Han S, Yin G, Tu K (2017) MiR-324-3p promotes tumor growth through targeting DACT1 and activation of Wnt/ β -catenin pathway in hepatocellular carcinoma. *Oncotarget* 8(39):65687
38. Yuan G, Wang C, Ma C, Chen N, Tian Q, Zhang T, Fu W (2012) Oncogenic function of DACT1 in colon cancer through the regulation of beta-catenin. *PLoS One* 7(3):e34004
39. Mercer TR, Dinger ME, Mattick JS (2009) Long non-coding RNAs: insights into functions. *Nat Rev Genet* 10(3):155–159
40. Ma H, Chang H, Yang W, Lu Y, Hu J, Jin S (2020) A novel IFN α -induced long noncoding RNA negatively regulates immunosuppression by interrupting H3K27 acetylation in head and neck squamous cell carcinoma. *Mol Cancer* 19(1):4
41. Barnes CE, English DM, Cowley SM (2019) Acetylation & Co: an expanding repertoire of histone acylations regulates chromatin and transcription. *Essays Biochem* 63(1):97–107
42. Liu X, Lu Y, Zhu J, Liu M, Xie M, Ye M, Li M, Wang S, Ming Z, Tong Q, Liu F, Zhou R (2019) A Long Noncoding RNA, Antisense IL-7, Promotes Inflammatory Gene Transcription through Facilitating Histone Acetylation and Switch/Sucrose Nonfermentable Chromatin Remodeling. *J Immunol* 203(6):1548–1559
43. Dong H, Wang W, Mo S, Chen R, Zou K, Han J, Zhang F, Hu J (2018) SP1-induced lncRNA AGAP2-AS1 expression promotes chemoresistance of breast cancer by epigenetic regulation of MyD88. *J Exp Clin Cancer Res* 37(1):202
44. Postepska-Igielska A, Giwojna A, Gasri-Plotnitsky L, Schmitt N, Dold A, Ginsberg D, Grummt I (2015) LncRNA Khps1 regulates expression of the proto-oncogene SPHK1 via triplex-mediated changes in chromatin structure. *Mol Cell* 60(4):626–636
45. Khilji S, Hamed M, Chen J, Li Q (2018) Loci-specific histone acetylation profiles associated with transcriptional coactivator p300 during early myoblast differentiation. *Epigenetics* 13(6):642–654

Figures

Figure 1

Overexpression of CRNED hinders the progression of post-traumatic osteoarthritis in rats. An OA rat model was established by using anterior cruciate ligament transectomy. Ad-CRNED or adenovirus empty vector were injected into OA rat model via tail vein. Four weeks after the operation, (a) the expression of CRNED was detected by using qPCR. b HE staining was performed to analyze the pathological changes of knee joints in rats. c Safranin-O-fast green staining was used to evaluate the pathological changes of the medial tibial plateau joint in rats. d Osteoarthritis Research Society International (OARS) scores. e The expression of Collagen 2 and Aggrecan in knee articular cartilage was measured with immunohistochemistry. f TUNEL staining was conducted to assess cell apoptosis. N=8 in each group. *P < 0.05, **P < 0.01.

Figure 2

Protective effects of CRNED on IL-1 β -induced apoptosis, inflammation and ECM degradation in chondrocyte-like ATDC5 cells. Ad-CRNED was transfected into chondrocyte-like ATDC5 cells for 24 h, and then IL-1 β (10 ng/mL) was used to treat cells for 24 h. a CRNED expression was measured. b Cell proliferation was detected by using MTT assay. c Flow cytometry was performed to analyze apoptosis. d The concentrations of IL-6, IL-8 and TNF- α were determined by ELISA. e The protein levels of MMP13, ADAMTS-5, Collagen 2 and Aggrecan were measured by using Western blotting. N=5 in each group. *P < 0.05, **P < 0.01.

Figure 3

DACT1 was a downstream target of CRNED. a The mRNA and protein levels of DACT1 in the OA rat model were assessed. b The mRNA and protein levels of DACT1 in IL-1 β -stimulated chondrocyte-like ATDC5 cells were detected. c RIP assay was performed to confirm the binding relationship between CRNED and DACT1. d RNA pull down was carried out to verify the binding of CRNED and DACT1 by using CRNED probe. e, f Ad-CRNE, sh-CRNE or their respective controls were transfected into chondrocyte-like ATDC5 cells, and the mRNA and protein levels of DACT1 were analyzed. N=5 in each group. *P < 0.05.

Figure 4

Silencing DACT1 resisted the protective effect of CRNE on IL-1 β -induced cell damage. Ad-CRNE was transfected into chondrocyte-like ATDC5 cells alone or together with sh-DACT1 for 24 h, and then IL-1 β

(10 ng/mL) was used to treat cells for 24 h. a, b The mRNA and protein expression of DACT1 was detected. c Cell proliferation was detected by using MTT assay. d Flow cytometry was performed to analyze apoptosis. e The concentrations of IL-6, IL-8 and TNF- α were determined by ELISA. f The protein levels of MMP13, ADAMTS-5, Collagen 2 and Aggrecan were measured. N=5 in each group. *P < 0.05.

Figure 5

CRNED promoted DACT1 expression by modulating H3K27 acetylation. a Bioinformatics analysis showed that H3K27ac was highly enriched in the DACT1 promoter region. b The expression of CRNDE in

the nucleus and cytoplasm was evaluated. U6 and GAPDH were used as nuclear and cytoplasmic markers, respectively. c ChIP assay showed the enrichment of H3K27ac in the DACT1 promoter region. d Chondrocyte-like ATDC5 cells were treated by IL-1 β for 24 h, and then the the enrichment of H3K27ac in the DACT1 promoter was analyzed by using ChIP assay. e, f After overexpression or interference with CRNED in chondrocyte-like ATDC5 cells, the enrichment of H3K27ac in DACT1 promoter was detected. N=5 in each group. *P < 0.05, **P < 0.01.

Figure 6

CRNED mediated H3K27 acetylation at DACT1 promoter region by binding to p300. a, b Ad-p300, sh-p300 or their respective controls were transfected into chondrocyte-like ATDC5 cells, and the mRNA and protein levels of DACT1 were detected. c ChIP assay showed the enrichment of p300 in the DACT1 promoter region. d RIP assay was performed to determine the binding between CRNED and DACT1. e After IL-1 β treatment, RIP assay was used to measure the binding of CRNED and p300 in chondrocyte-like ATDC5 cells. f, g After overexpression or interference with p300 in chondrocyte-like ATDC5 cells, the enrichment of H3K27ac in DACT1 promoter was analyzed. h, i After overexpression or interference with CRNED in chondrocyte-like ATDC5 cells, the enrichment of p300 in DACT1 promoter was assessed. g, h The mRNA and protein levels of p300 were determined after overexpressing or inhibiting CRNED in chondrocyte-like ATDC5 cells. N=5 in each group. *P < 0.05, **P < 0.01.

Figure 7

CRNED restrained Wnt/ β -catenin pathway activation by up-regulating DACT1 expression in IL-1 β -treated chondrocyte-like ATDC5 cells. a, b Ad-DACT1, sh-DACT1 or their respective controls were transfected into chondrocyte-like ATDC5 cells, and the protein levels of nuclear β -catenin, and c-Myc and Cyclin D1 were measured. c, d Ad-CRNED was transfected into cells alone or together with sh-DACT1 for 24 h, and then IL-1 β was used to stimulate cells for 24 h. The expression of β -catenin, c-Myc and Cyclin D1 was evaluated. e, f Chondrocyte-like ATDC5 cells were treated by IL-1 β alone or together with Ad-CRNED, and

then cells were treated by HY-122816. The expression of β -catenin, c-Myc and Cyclin D1 was analyzed. N=5 in each group. *P < 0.05.

Figure 8

CRNE slowed the progression of post-traumatic OA in rats by modulating DACT1. An OA rat model was established by using anterior cruciate ligament transectomy. Ad-CRNE was injected into the OA rat model alone or together with sh-DACT1 via tail vein. Four weeks after the operation, a the protein level of

DACT1 was detected. b HE staining was used to assess the pathological changes of knee joint tissues in rats. c Safranin-O-fast green staining was performed to evaluate the pathological changes of the medial tibial plateau joint in rats. d Osteoarthritis Research Society International (OARSI) scores. e TUNEL staining was performed to analyze cell apoptosis. N=8 in each group. *P < 0.05.