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Original Article

LncRNA CRNDE regulates the differentiation of tendon-derived stem cells and enhances rotator cuff injury repair by modulating the miR-337/TGFBR2 axis

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Abstract: The differentiation of stem cells into tendon cells plays a crucial role in the repair of rotator cuff injuries. Long non-coding RNAs (lncRNAs) are known to regulate tendon-derived stem cell (TDSC) differentiation during tendon injury; however, the specific mechanisms involving the lncRNA colorectal neoplasia differentially expressed (CRNDE) have not been fully elucidated. In this study, we successfully isolated and cultured TDSCs, and established a tenogenic differentiation model through ascorbic acid treatment. RNA sequencing analysis showed that ascorbic acid significantly upregulated CRNDE expression. Furthermore, CRNDE overexpression in TDSCs markedly enhanced tenogenic differentiation. Using bioinformatics analysis in combination with luciferase reporter assays, we demonstrated that CRNDE and transforming growth factor beta receptor 2 (TGFBR2) contain shared binding sequences for miR-337, suggesting a competitive regulatory interaction. Overexpression of miR-337 was found to inhibit CRNDE-induced tenogenic differentiation and reduce both TGFBR2 mRNA and protein levels. In contrast, CRNDE knockdown decreased TGFBR2 protein expression and impaired tenogenic differentiation of TDSCs. In a rat model of rotator cuff tear (RCT), transplantation of CRNDE-overexpressing TDSCs significantly enhanced functional recovery, an effect associated with upregulation of TGFBR2. Taken together, these results demonstrate that CRNDE promotes tenogenic differentiation and tendon-bone healing through modulation of the miR-337/TGFBR2 signaling axis.

Keywords: Rotator cuff tear, lncRNA, miRNA-337, TGFBR2, tendon-derived stem cells

Introduction

Tendon-bone healing plays a critical role in the recovery from rotator cuff tears (RCTs). The tendon-bone junction is a complex, multi-tissue interface composed of tendons, fibrocartilage, and bone, which presents significant challenges for effective integration following injury [1]. Currently, healing often results in the formation of granulation tissue rather than the fibrocartilage essential for proper tendon insertion, leading to suboptimal outcomes [2]. High postoperative re-tear rates suggest that conventional repair techniques may not sufficiently facilitate tendon-bone integration, underscoring the need for further investigation into more effective therapeutic strategies [3]. To enhance tendon-bone healing and improve surgical results, various biological approaches - such as the use of growth factors, stem cells, and biomaterials - have been explored [4, 5]. Research indicates that calcium phosphate-based materials can promote new bone formation and improve collagen organization at the tendon-bone interface, potentially enhancing healing outcomes [6]. Additionally, mesenchymal stem cells (MSCs) have been associated with improved tendon-bone healing, with studies showing a notable decrease in MSC content in patients suffering from rotator cuff tears [7]. Ensuring robust initial fixation and mechanical stability at the tendon-bone interface is crucial for successful rotator cuff repair [8]. Recent advances in surgical techniques and a deeper understanding of rotator cuff biology have led to the development of novel strategies designed to enhance mechanical stability and promote tissue integration at the repair site.

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Various biological treatments, including mesenchymal stem cells and growth factors, are currently under investigation to enhance tendon healing outcomes [9, 10]. The combination of bone marrow aspirate concentrate (BMAC) and platelet-rich plasma has been found to stimulate the proliferation and migration of tendon-derived stem cells (TDSCs), offering potential improvements in the treatment of rotator cuff injuries [11]. Injectable hydrogels incorporating bioactive agents have also shown promise in supporting the regeneration of the tendon-bone junction, representing a novel and effective therapeutic strategy [12]. TDSCs are increasingly recognized as a valuable cell source for tendon repair and regeneration due to their inherent stem cell characteristics and capacity to promote tenogenic differentiation [13, 14]. Their behavior can be modulated by external factors, such as exosomes derived from adipose stem cells, which have demonstrated the ability to enhance TDSC proliferation and improve rotator cuff healing [15]. Consequently, combining tissue engineering strategies with stem cell therapy holds significant potential for overcoming the challenges associated with tendon repair [10].

Research into the roles of long non-coding RNAs (lncRNAs) in rotator cuff tendinopathy has revealed promising therapeutic targets, underscoring the critical importance of elucidating molecular interactions in tendon pathology. LncRNA H19 has been shown to enhance tenogenic differentiation and promote tendon healing by targeting miR-140-5p and upregulating VEGFA expression, suggesting its potential as a therapeutic candidate for tendon injuries [14]. Another study further demonstrated that H19 facilitates tenogenic differentiation through activation of the TGF-β1 signaling pathway and by modulating miR-29b-3p, thereby establishing a positive feedback loop that supports tendon repair [16]. Additionally, X-inactive specific transcript (XIST) has been implicated in tendon adhesion and injury, where it promotes fibroblast proliferation via the miR-26a-5p/COX2 pathway, highlighting its involvement in tendon healing mechanisms [17]. Meanwhile, MALAT1 plays a crucial role in regulating the tenogenic differentiation of tendon-derived stem cells (TDSCs) through the miR-378a-3p/MAPK1 axis, further emphasizing its relevance in the recovery process from tendon injuries [18].

This study aims to elucidate the role and underlying mechanism of the long non-coding RNA-CRNDE in regulating the miR-337/TGFBR2 signaling axis during tendon stem cell differentiation and tendon injury repair, using both a rat rotator cuff injury model and *in vitro* tendon stem cell experiments, with the goal of providing novel insights and potential therapeutic targets for the clinical management of tendon injuries.

Materials and methods

Animals and ethics

The Sprague-Dawley (SD) rats used in this study were purchased from the Laboratory Animal Center of Guangdong, China. All animal procedures were reviewed and approved by the Ethics Committee of Sun Yat-sen University (Shenzhen, Guangdong, China) and conducted in accordance with the institution's guidelines for the care and use of laboratory animals.

Isolation and identification of TDSCs

Tendon-derived stem cells (TDSCs) were isolated from the Achilles tendons of Sprague-Dawley rats using a previously established protocol [19]. In brief, the tendons were digested with type I collagenase (Sigma-Aldrich, St. Louis, MO, USA) for 2 hours at 37°C. The resulting cell suspension was then seeded into 100 mm culture dishes at a density of 200 cells/cm^2 and cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Scientific, Waltham, USA), supplemented with 20% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin (Gibco), and 100 U/ml streptomycin (Gibco), under standard incubation conditions for 10 days. Cells from passages 3 to 4 were used for subsequent analyses. The identity of TDSCs was confirmed using flow cytometry with the appropriate antibodies, based on established criteria [19].

Trilineage differentiation of TDSCs

Trilineage differentiation of TDSCs was conducted using a previously established protocol [20]. To evaluate their differentiation potential, adipogenic and osteogenic differentiation kits were employed. In summary, TDSCs were seeded into 48-well plates at a density of 5×10^4 cells per well and cultured in either adipogenic or osteogenic induction media. Following 10 to 14 days of incubation, the cells were fixed

with 4% paraformaldehyde (#P0099; Beyotime, Shanghai, China) for 20 minutes and subsequently stained with Oil Red O (#C0157S; Beyotime) and Alizarin Red (#C0138; Beyotime), in accordance with the manufacturer's instructions.

Construction of stable TDSCs with CRNDE overexpression

To generate a stable TDSC cell line overexpressing CRNDE, CRNDE fragments were cloned into a lentiviral vector. Lentiviral particles were subsequently produced by transient transfection of the recombinant vector into HEK293 cells. The viral supernatants were harvested and cleared of cellular debris by filtration through 0.45 μm Millex-HV filters (Millipore, Bedford, MA, USA). TDSCs were then transduced with either control lentivirus or CRNDE-overexpressing lentivirus to establish stable cell lines. Forty-eight hours after infection, the cells were subjected to puromycin selection (#ST551-10mg; Beyotime), and the efficiency of CRNDE overexpression was validated by qRT-PCR analysis.

Cell transfection

The Rno-miR-337-3p mimic (5'-UUCAGCUCCUAUAUGAUGCCUUU-3'), Rno-miR-337-3p inhibitor (5'-AAAGGCAUCAUAUAGGAGCUG-3'), and their respective negative controls (NCs) (5'-UUCUCCGAACGUGUCACGUTT-3'), were obtained from GenePharma (Shanghai, China). Transfection was performed using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

Luciferase reporter assay

Luciferase reporter constructs for CRNDE and TGFBR2 were generated by inserting the respective sequences into the pGL3 vector (Promega, Madison, WI, USA). Both wild-type (WT) and mutant (MUT) versions of CRNDE and TGFBR2, containing the miR-337-3p binding site, were cloned into the vector. The constructs were then co-transfected with a Renilla luciferase-expressing internal control plasmid into 293T cells using the Dual-Luciferase Reporter Assay System (Promega), according to the manufacturer's instructions. In addition, cells were transfected with either a miR-337-3p mimic, a miR-337-3p inhibitor, or a negative control (NC) fragment. Following a 48-hour incubation period, cells were collected, and luciferase activity was assessed using the Luciferase Assay System (Promega), with results normalized to the Renilla luciferase signal.

Sirius red staining

A Sirius Red staining assay was conducted to assess collagen formation in TDSCs. Briefly, TDSCs were seeded into a 12-well plate. Following removal of the culture medium, the cells were rinsed and fixed with 4% paraformaldehyde for 40 minutes. Subsequently, the cells were incubated with 0.3% Sirius Red solution prepared in saturated picric acid (Sigma-Aldrich, MA, USA) for 45 minutes.

qRT-PCR

Total RNA was extracted from all experimental conditions using TRIzol reagent (#R0016; Beyotime, Shanghai, China) according to the manufacturer's instructions. First-strand cDNA synthesis was performed using random primers (Invitrogen, USA). Real-time quantitative reverse transcription PCR (qRT-PCR) was carried out with the ABI Prism 7000 sequence detection system (Applied Biosystems) and SYBR Green PCR Master Mix (Applied Biosystems, USA). The primer sequences used in the reactions are provided in **Table 1**. Relative mRNA expression levels were normalized to GAPDH as an internal control.

Western blotting

Cell lysates were prepared and lysed on ice using Laemmli buffer (#FD010; Beyotime) [21]. The lysates were separated by 8% SDS-PAGE and subsequently transferred onto nitrocellulose membranes. Membranes were blocked with 5% nonfat milk in PBS for 1 hour at room temperature, followed by overnight incubation at 4°C with primary antibodies diluted at a ratio of 1:1000 (TGFBR2, #ab269279, Abcam, Cambridge, UK; GAPDH, #AF0006; Beyotime). After a brief rinse with PBS containing 0.1% Tween-20, the membranes were incubated with the corresponding HRP-conjugated secondary antibodies. Protein bands were detected using BeyoECL Plus reagent (#P0018S; Beyotime), and the relative band intensities were analyzed using ImageJ software (Bethesda, MD, USA).

Table 1. Primer sequences for real-time qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
CRNDE	CGCGCCCGCGCGGCGGAGGA	AGTATGAATTGCAGACTTTGCA
Scx	AGCCCAAACAGATCTGCACCTT	CTTCCACCTTCACTAGTGGCATCA
Mkx	CTCAAGGACAACCTCAGCCTGAG	CGTTGCCCTGAACATACTTGTTGTAC
Tnmd	ATGGGTGGTCCCACAAGTGAA	CTCTCATCCAGCATGGGATCAA
Col1a1	GTCCGAGGTCCTAATGGAGATGC	GGTCCAGGGAATCCGATGT
Col2a1	CCAGGTCCTGCTGGAAAA	CCTCTTTCTCCGGCCTTT
Fmod	CAAGGCAACAGGATCAATGAG	CTGCAGCTTGGAGAAGTTCA
Dcn	GACTCCACGACAATGAGATCACC	GTTGCCATCCAGATGCAGTTC
TGFBR2	CAAGTCGGATGTGGAAATGG	AAATGTTTCAGTGGATGGATGG
Gapdh	TGATTCTACCCACGGCAAGTT	TGATGGGTTTCCCATTGATGA
miR-337	CGGCGTCATGCAGGAGTTG	GAACATGTCTGCGTATCTC
miR-217	ACTGCATCAGGAACTGAC	GAACATGTCTGCGTATCTC
miR-181a	AACATTCAACGCTGTCGGTG	GAACATGTCTGCGTATCTC
miR-136	ACTCCATTTGTTTTGATGATG	GAACATGTCTGCGTATCTC
miR-126	CATTATTACTTTTGGTACGCG	GAACATGTCTGCGTATCTC

Rat RCT model and biomechanical testing

To establish a rat rotator cuff tear (RCT) model, the method was employed as previously described [14]. A longitudinal incision approximately 1.5 cm in length was made on the anterolateral aspect of the shoulder to expose the supraspinatus tendon and muscle. Half of the tendon was then transected and sharply detached from the greater tuberosity. The tendon defect was repaired using a layered 4-0 suture technique. The rats were randomly assigned to three groups: control, TDSCs + control, and TDSCs + CRNDE (containing CRNDE-overexpressing stable TDSCs), with eight rats in each group. In the control group, 200 μL of fibrin glue was injected at the tendon-bone interface. In the TDSC-treated groups, either 2×10^6 normal TDSCs or CRNDE-overexpressing TDSCs were suspended in fibrin glue and administered at the same site. Biomechanical testing of the tendons was conducted at four and eight weeks post-surgery.

For biomechanical testing, the entire supraspinatus tendon along with the proximal humerus was harvested from each rat. The specimens were tested using an MTS 858 materials testing system (MTS Systems Corporation, Minneapolis, MN, USA). A preload ranging from 0 to 5 N was applied, and the tendons were then subjected to tensile loading at a crosshead speed of 14 mm/s. The ultimate failure force (N) and stiffness (N/mm) were recorded. Ultimate failure force was determined as the peak load from the load-displacement curve, while stiffness was calculated as the slope of the linear portion of the curve. Data were analyzed using SigmaPlot 8.0 software (Sigma-Aldrich). Following biomechanical testing, the animals were anesthetized with 4% isoflurane and subsequently euthanized. Tissue samples were then collected for subsequent experiments, including qRT-PCR analysis.

Data analysis

Statistical analysis and data visualization were performed using GraphPad Prism (Version 10.0; San Diego, CA, USA). The results are expressed as the mean ± standard deviation (SD) from three independent experiments. Data normality was evaluated using the D'Agostino-Pearson test. Group comparisons were conducted using one-way or two-way analysis of variance (ANOVA), as appropriate. Post hoc comparisons following one-way ANOVA were carried out using Bonferroni's test, whereas Tukey's test was applied for multiple comparisons in two-way ANOVA. For comparisons between two groups, an independent Student's *t*-test was used. Statistical significance was defined as $P < 0.05$.

Results

TDSCs characterization

TDSCs were isolated as described in the Materials and Methods section. The cells were

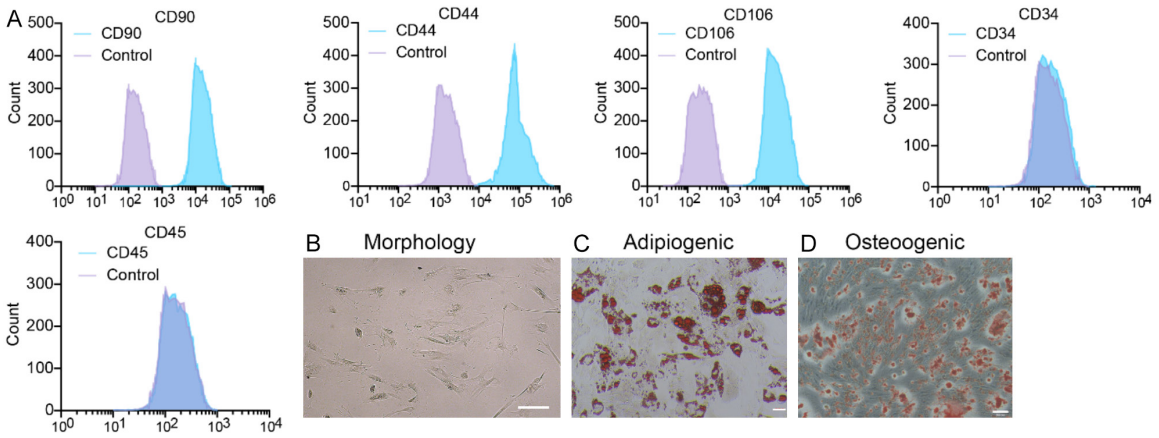

Figure 1. Characterization of TDSCs. A. TDSCs were positive for CD90, CD44, and CD106 but negative for CD34 and CD45. B. Cell morphology of TDSCs. C, D. Adipogenesis and osteogenesis are revealed by Oil red O and Alizarin red S staining. Scale bar: 20 μm.

obtained from the Achilles tendon of rats and cultured across several passages. To confirm the identity of the cultured cells as TDSCs, their surface markers were analyzed using flow cytometry. As shown in **Figure 1A**, the results revealed a clear expression of the mesenchymal stem cell markers CD90, CD44, and CD106, while showing no expression of the endothelial and hematopoietic markers CD34 and CD45. TDSCs at passage 3 (P3) exhibited a consistent, elongated spindle-shaped morphology, characteristic of tendon-derived stem cells (**Figure 1B**). To evaluate their differentiation potential, TDSCs were subjected to in vitro induction, and the results demonstrated successful differentiation into both adipogenic (**Figure 1C**) and osteogenic (**Figure 1D**) lineages.

CRNDE is upregulated during tenogenic differentiation of TDSCs

To induce tenogenic differentiation, TDSCs were treated with ascorbic acid (AA), as previously described [22, 23]. Subsequently, cell lysates were collected from both the control and ascorbic acid-treated groups, each with four replicates, and subjected to RNA sequencing (not all raw data were shown). Differentially expressed genes were then analyzed. As shown in **Figure 2A**, the heatmap revealed that the lncRNA CRNDE was significantly upregulated in the ascorbic acid-treated group. These findings were further validated by qRT-PCR, which confirmed that CRNDE expression was notably increased during ascorbic acid-induced tenogenic differentiation of TDSCs (**Figure 2B**). To further investigate the role of CRNDE in tenogenic induction, a stable TDSC cell line overexpressing CRNDE was established using a lentiviral system. As demonstrated in **Figure 2C**, CRNDE overexpression markedly enhanced collagen formation under ascorbic acid treatment, as evidenced by Sirius red staining. Additionally, the mRNA expression levels of key tenogenic and extracellular matrix markers, including Scx, Mkx, Col1a1, Fmod, Tnmd, and Dcn, were evaluated by qRT-PCR. As shown in **Figure 2D**, these genes were significantly upregulated in the CRNDE-overexpressing TDSCs. Collectively, these results indicate that CRNDE is upregulated during the tenogenic differentiation of TDSCs.

CRNDE sponges miR-337

We next sought to investigate the regulatory mechanism by which CRNDE influences the tenogenic differentiation of TDSCs. Based on the RNA-seq results, we identified several CRNDE-associated miRNAs, including miR-217, miR-181a-5p, miR-136, miR-126-5p, and miR-337. To validate their expression levels, we performed qRT-PCR analysis. Among these miRNAs, only miR-337 exhibited a significant decrease in expression during tenogenic induction by AA (**Figure 3A**). Using bioinformatics tools such as TargetScan and StarBase-ENCORI [24], we predicted a complementary binding site for miR-337 within the 3'UTR region of CRNDE, suggesting a potential regulatory interaction between CRNDE and miR-337 (**Figure 3B**). To confirm direct regulation, we conducted a dual-luciferase reporter assay by cloning both the wild-type (WT) and mutant (MUT) 3'UTR

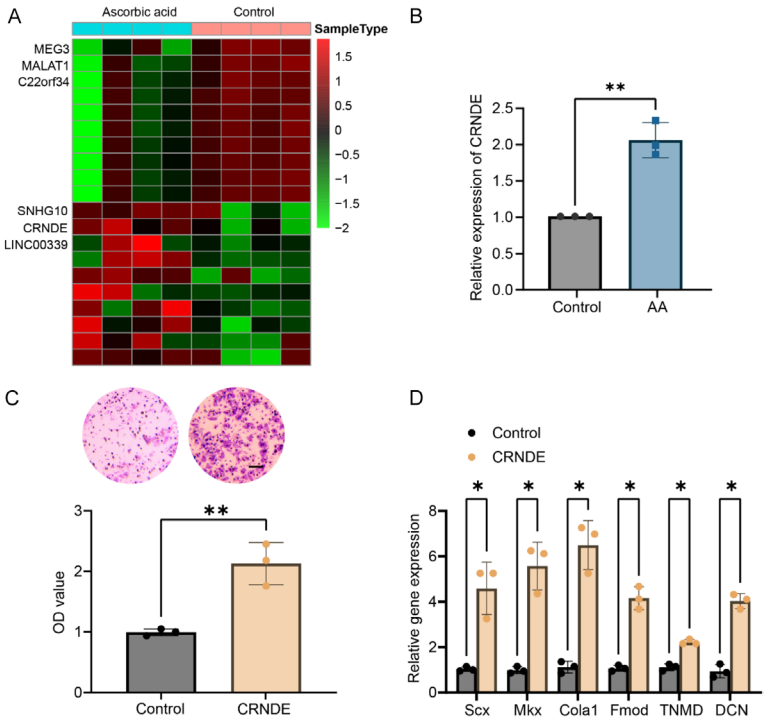

Figure 2. CRNDE is upregulated during tenogenic differentiation. A. Heatmap showing upregulated and downregulated genes in the control group versus the ascorbic acid-induced group. B. qRT-PCR analysis confirmed the increased expression of CRNDE. C. Overexpression of CRNDE in TDSCs promoted tenogenic differentiation. Scale bar: 20 μm. D. qRT-PCR results demonstrating the mRNA levels of various tenogenic markers. *P < 0.05; **P < 0.01. Scale bar: 20 μm.

sequences of CRNDE into a reporter vector (**Figure 3B**). The results demonstrated that transfection with miR-337 mimics significantly reduced the luciferase activity of the WT reporter, but not the MUT counterpart (**Figure 3C**). Moreover, overexpression of miR-337 effectively reversed the enhancing effect of CRNDE overexpression on tenogenic differentiation, as evidenced by Sirius red staining (**Figure 3D**, **3E**). Collectively, these findings indicate that CRNDE acts as a sponge for miR-337 during tenogenic differentiation.

CRNDE modulates the miR-337/TGFBR2 axis during the tenogenic differentiation of TDSCs

We next aimed to identify the downstream target of miR-337 by combining RNA-seq data analysis with the previously described bioinformatics tools. TGFBR2 was identified as a promising candidate due to the presence of multiple miR-337 binding sites (highlighted in red, **Figure 4A**). To validate this interaction, both the wild-type (WT) 3'UTR of TGFBR2 containing the binding sites and a corresponding mutant version (MUT, highlighted in green; **Figure 4A**) were cloned into luciferase reporter vectors. As shown in **Figure 4B**, transfection with a miR-337 mimic significantly reduced luciferase activity in cells carrying the WT construct, whereas no such effect was observed in those with the MUT construct. Furthermore, in cultured TDSCs, the miR-337 mimic decreased, while the miR-337 inhibitor increased, both the mRNA and protein expression levels of endogenous TGFBR2 (**Figure 4C-E**). These results confirm that TGFBR2 is a direct target of miR-337, which finding is consistent with earlier reports [25]. To determine whether CRNDE regulates tenogenic differentiation through the miR-337/TGFBR2 axis, we assessed the effects of miR-337 inhibition. As illustrated in **Figure 5A**, **5B**, overexpression of the miR-337 inhibitor markedly increased TGFBR2 protein levels in TDSCs; however, this enhancement was diminished by CRNDE knockdown. Moreover, the miR-337 inhibitor promoted tenogenic differentiation, an effect that was reversed upon co-transfection with shCRNDE (**Figure 5C**, **5D**). Together, these findings indicate that CRNDE modulates the tenogenic differentiation of TDSCs via the miR-337/TGFBR2 signaling pathway.

CRNDE overexpression enhances the recovery of RCT

We examined whether CRNDE overexpression enhances functional recovery in a rotator cuff tear (RCT) model. The RCT model was established as described in the Materials and Methods section, and animals were treated with either vehicle control, control TDSCs, or CRNDE-overexpressing TDSCs at the surgical site. Biomechanical testing performed at 4 and 8 weeks post-treatment showed a significant increase in ultimate load in the CRNDE-overexpressing TDSC group compared to control groups at both time points (**Figure 6A**). Stiffness was significantly improved only at the 8-week

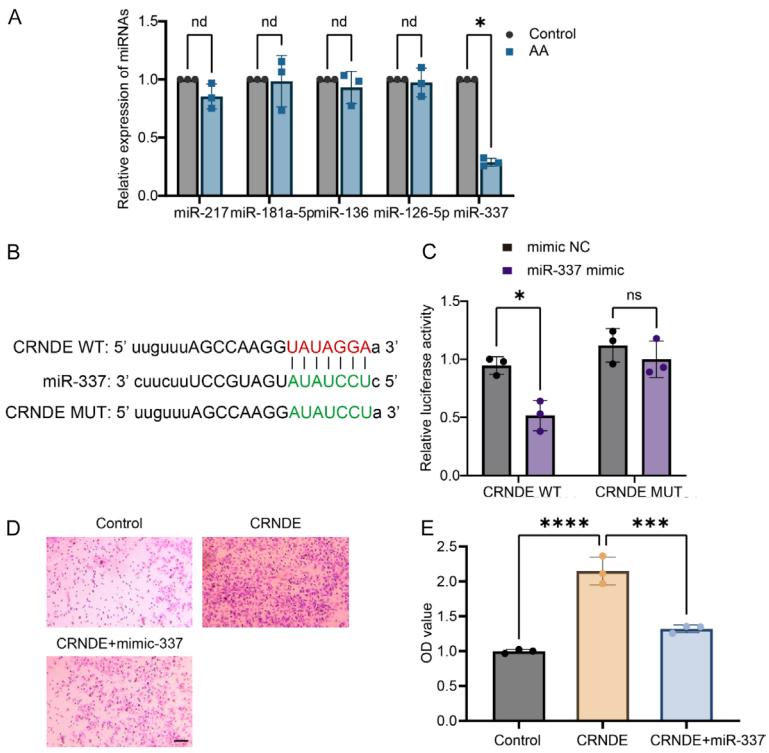

Figure 3. CRNDE acts as a sponge for miR-337. A. qRT-PCR analysis showing the expression levels of various miRNAs during AA-induced tenogenic differentiation. B. Bioinformatic prediction of potential binding sites of CRNDE for miR-337. C. Luciferase reporter assay confirming the interaction between CRNDE and miR-337. D. Sirius red staining illustrating tenogenic induction following AA treatment under different experimental conditions. E. Quantitative analysis of Sirius red staining results. nd/ns, not significant; *P < 0.05; ***P < 0.001; ****P < 0.0001. Scale bar: 20 μm.

time point, with no marked difference observed at 4 weeks (**Figure 6B**). Furthermore, qRT-PCR analysis of tendon tissues revealed increased mRNA expression of TGFBR2 and Col1A1 in both TDSC-treated groups, with the CRNDE-overexpressing group showing even greater upregulation (**Figure 6C**, **6D**). These findings indicate that CRNDE overexpression significantly enhances the therapeutic effects of TDSC transplantation, thereby promoting tendon-bone healing in the RCT model.

Discussion

Tendon-Derived Stem Cells (TDSCs) play a critical role in the repair of rotator cuff tears by promoting tendon healing and regeneration, although the precise mechanisms involved are not yet fully understood. In this study, we successfully isolated and characterized TDSCs from rat Achilles tendons and established an ascorbic acid-induced model of tenogenic differentiation. Long non-coding RNA CRNDE was found to be significantly upregulated during tenogenic differentiation, and its overexpression was shown to markedly enhance this process. Mechanistically, miR-337 was identified as a direct target of CRNDE, and TGFBR2 was confirmed to be a downstream target of miR-337 in TDSCs. Our findings indicate that CRNDE promotes tenogenic differentiation through regulation of the miR-337/TGFBR2 axis. Furthermore, *in vivo* experiments demonstrated that CRNDE overexpression significantly improved functional recovery in a rat model of rotator cuff tear, underscoring its therapeutic potential for tendon repair.

The regulation of tendon-bone healing in rotator cuff tears by TDSCs is a complex and multi-layered process, involving their differentiation into tenogenic lineages, secretion of growth factors, and modulation of the local cellular microenvironment. TDSCs release exosomes enriched with vascular endothelial growth factor A, which promotes tenocyte differentiation, migration, and transition to a fibroblastic phenotype - key events in tendon repair [26]. Exosomes derived from adipose-derived stem cells have also been shown to modulate TDSC behavior, thereby enhancing rotator cuff repair, suggesting a synergistic interaction in promoting tissue healing [15]. Additionally, the combination of bone marrow aspirate concentrate and platelet-rich plasma (BMAC-PRP) with TDSCs has been reported to enhance their proliferation and migration, which is beneficial for tendon regeneration [11]. Encapsulation of TDSCs in chitosan hydrogel can further support tendon-to-bone healing by upregulating tendon- and bone-related genes and improving the structural integrity of the repair site [27]. Genetic modulation of key factors such as S100A1 and RASSF8 has also been shown to enhance TDSC proliferation and improve outcomes in

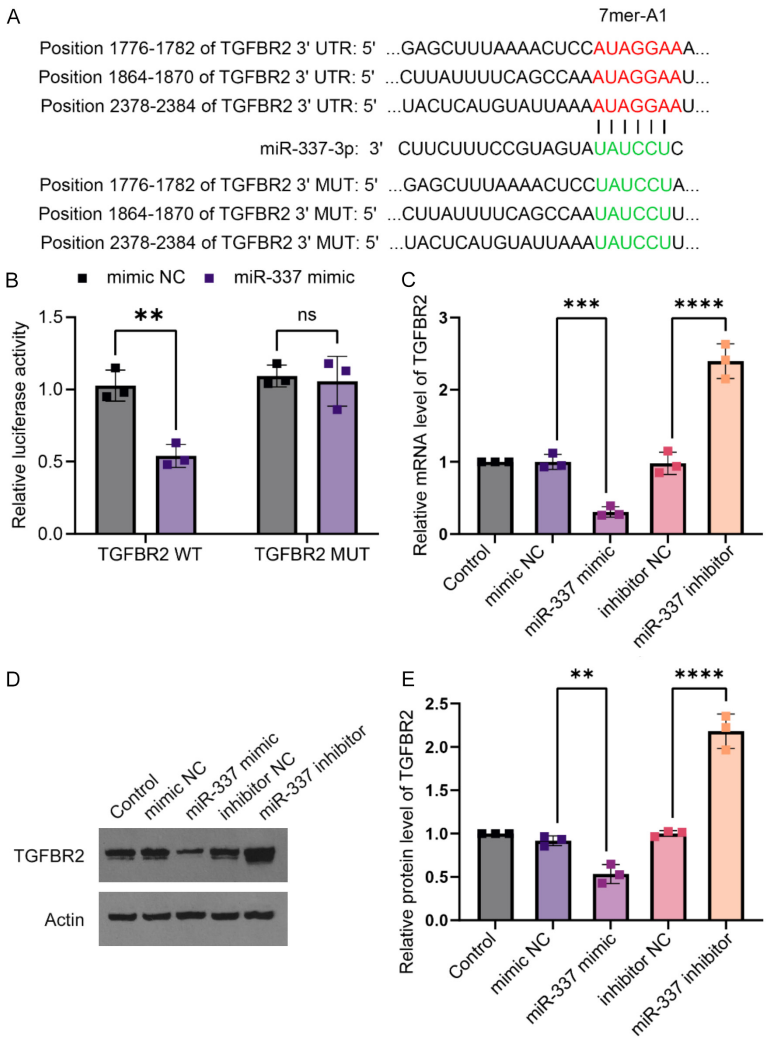

Figure 4. miR-337 directly targets TGFBR2. (A) Bioinformatics analysis predicting the binding sites of miR-337 within the TGFBR2 3'UTR region. (B) Luciferase reporter assay confirming the interaction between miR-337 and TGFBR2. (C) mRNA expression and (D, E) protein levels of TGFBR2 in transfected TDSCs. ns, not significant; **P < 0.01; ***P < 0.001; ****P < 0.0001.

rotator cuff healing [28]. Moreover, TDSCs contribute to improved structural and mechanical properties at the tendon-bone interface, a process mediated in part by tumor necrosis factor alpha-stimulated gene/protein 6 [29]. These cells possess the capacity for osteogenic and chondrogenic differentiation, an essential feature for effective tendon-bone junction healing, as demonstrated in this study [30]. However, it is important to note that the differentiation potential of TDSCs diminishes with age and the duration of injury, which may compromise their therapeutic efficacy in older individuals or those with chronic rotator cuff tears [31].

CRNDE, a long non-coding RNA, plays a pivotal role in bone injury and related disorders by modulating multiple cellular processes and signaling pathways. For instance, CRNDE is upregulated in osteosarcoma, a prevalent type of bone cancer, where it enhances cell proliferation, invasion, and migration by activating Notch1 signaling and facilitating epithelial-mesenchymal transition [32]. In the context of postmenopausal osteoporosis, CRNDE is involved in the regulation of osteoclast proliferation through the PI3K/Akt pathway. Estrogen deficiency, commonly observed in postmenopausal women, leads to elevated CRNDE expression, which in turn promotes bone resorption and contributes to bone loss [33]. Additionally, CRNDE influences osteoblast proliferation and differentiation via the Wnt/β-catenin signaling pathway, and its expression is essential for maintaining bone mass and supporting bone formation, underscoring its significance in bone repair and regeneration [34]. In osteoarthritis, CRNDE facilitates the chondrogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and contributes to cartilage repair by interacting with the SIRT1/SOX9 pathway, which is critical for cartilage matrix synthesis and stability [35]. Given its involvement in osteoarthritis, particularly in regulating inflammation and extracellular matrix (ECM) degradation, CRNDE may also have potential implications in tendon injury repair. In this study, we observed that CRNDE was significantly upregulated during tenogenic differentiation of TDSCs, and its overexpression promoted this process *in vitro*, as well as improved the recovery of rats with RCT injuries *in vivo*.

MiR-337 plays a crucial role in bone-related injuries by modulating key cellular processes

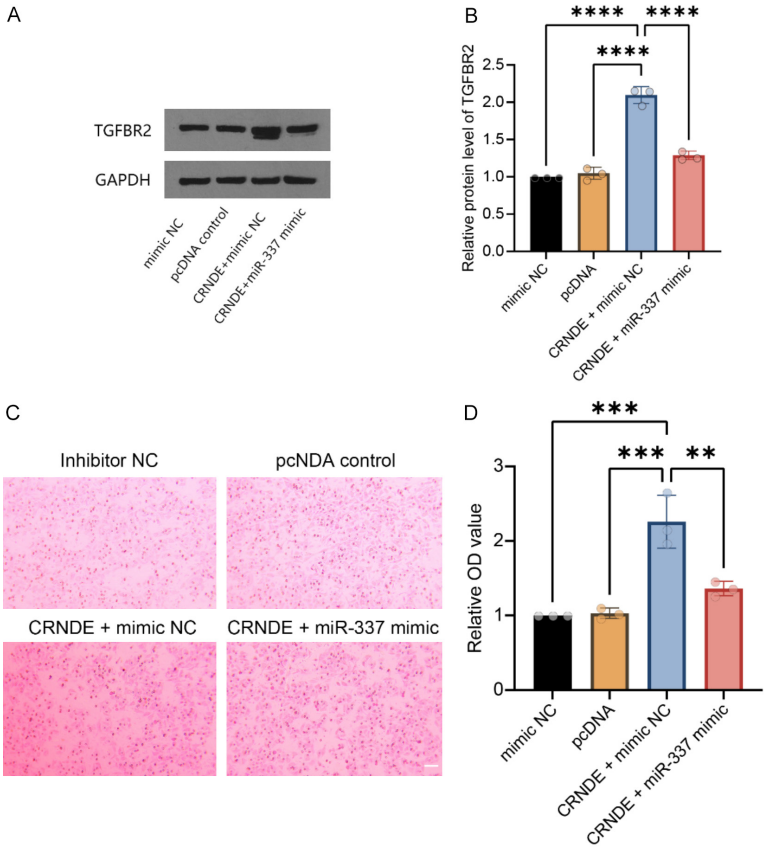

Figure 5. CRNDE regulates the miR-337/TGFBR2 axis during tenogenic differentiation. A, B. Protein expression levels of TGFBR2 were evaluated in TDSCs following transfection with CRNDE overexpression or miR-337 mimics. C, D. Tenogenic differentiation was assessed using Sirius red staining. **P < 0.01; ***P < 0.001; ****P < 0.0001. Scale bar: 20 μm.

such as proliferation, apoptosis, and differentiation. Specifically, miR-337-3p enhances chondrocyte proliferation and suppresses apoptosis through the regulation of the PTEN/AKT axis, a pathway centrally involved in the pathogenesis of osteoarthritis (OA) [34, 36]. In facet joint osteoarthritis (FJOA), miR-337-3p exerts protective effects by targeting SKP2, thereby inhibiting DUSP1 ubiquitination and inactivating the MAPK signaling pathway. This leads to reduced inflammation and tissue damage, underscoring its potential protective function in joint and bone injuries [37]. Furthermore, miR-337-5p, in conjunction with other microRNAs, promotes the proliferation and inhibits the apoptosis of osteoblast-like mesenchymal stem cells (OMMSCs), which are essential for bone repair and regeneration, highlighting its therapeutic potential in enhancing bone healing [38]. Conversely, miR-337 negatively regulates osteogenic differentiation in BMSCs by targeting Rap1A. This regulatory mechanism is particularly prominent under high-glucose conditions, which can compromise bone healing, indicating miR-337's involvement in bone metabolism and its potential relevance in the treatment of diabetic osteoporosis [39]. In addition to its role in bone health, miR-337 is implicated in tendon injury, particularly in tendinopathy associated with ectopic tendon ossification. MiR-337-3p is a mechanosensitive microRNA whose expression is reduced under mechanical loading in TDSCs. This downregulation is associated with the chondro-osteogenic differentiation of TDSCs, a key pathological process in tendinopathy [40]. Consistently, our findings also revealed a significant decrease in miR-337 expression during tenogenic differentiation. Notably, the long non-coding RNA CRNDE acts as a molecular sponge for miR-337, modulating this differentiation process and thereby promoting tendon-bone healing in rats with RCT.

TGFBR2, also known as transforming growth factor beta receptor 2, is a protein-coding gene that encodes a transmembrane serine/threonine kinase [41]. It forms a heterodimeric complex with TGF-beta receptor type-1 (TGFBR1) and facilitates the binding of TGF-beta ligands, thereby transducing extracellular signals into intracellular responses. This signaling cascade regulates a broad spectrum of physiological and pathological processes, such as cell proliferation, cell cycle arrest, wound healing, immunosuppression, and tumorigenesis [42]. Upon ligand binding, TGFBR2 phosphorylates TGFBR1, which subsequently activates SMAD2, leading to the modulation of transcription in TGF-beta-regulated genes [43]. TGFBR2 plays a pivotal role in the proliferation and differentiation of mesenchymal cells. It also contributes to the regulation of cell cycle arrest in epithelial and hematopoietic cells and supports the differentiation of osteogenic progenitor cells dur-

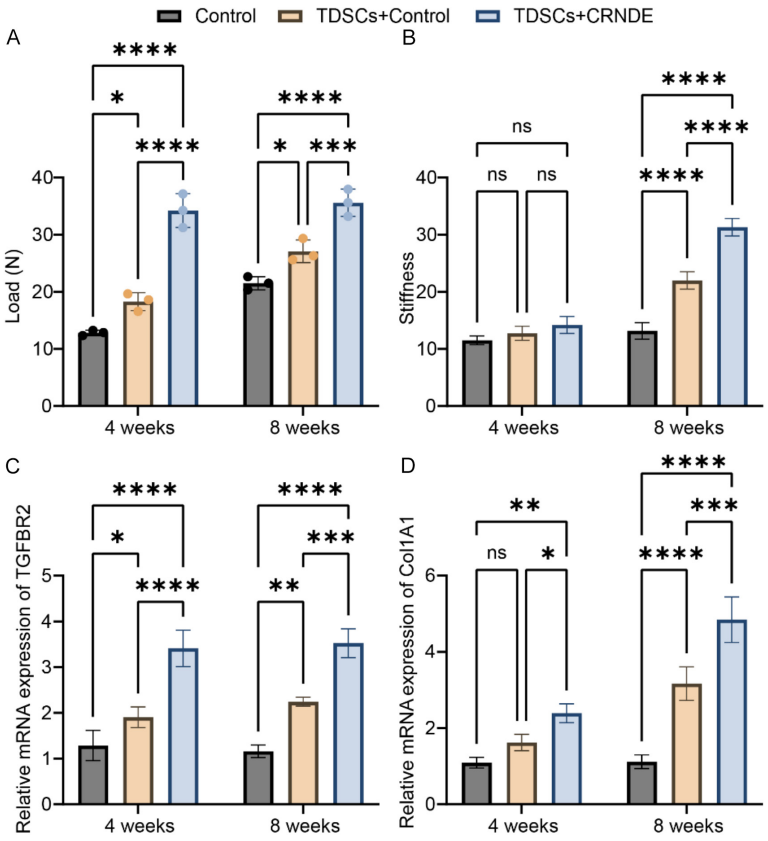

Figure 6. Overexpression of CRNDE enhances the recovery of RCT. RCT rats were injected with vehicle control, control TDSCs, or a stable TDSC cell line overexpressing CRNDE. (A) Ultimate load and (B) Stiffness were assessed through mechanical testing. (C, D) The mRNA expression levels of TGFBR2 and type I collagen (Col1A1) were quantified by qRT-PCR. ns, no significance; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

ing bone healing [44]. In the context of wound healing and tissue regeneration, TGFBR2 is essential for extracellular matrix formation and drives the differentiation of cells into chondrocytes and osteoblasts, both of which are critical for bone repair and regeneration [45]. Studies have shown that TGFBR2 signaling is indispensable for the proper development of skeletal structures; its deletion results in decreased osteoblast numbers and compromised bone volume [46, 47]. Moreover, TGFBR2 is involved in regulating chondrocyte differentiation, a key step in endochondral ossification, a process fundamental to bone repair [48]. The deletion of TGFBR2 specifically in chondrocytes leads to delayed differentiation and defective bone development [49]. In addition, TGFBR2 contributes to tenogenic differentiation, which is crucial for tendon repair. This process is mediated through crosstalk between integrin signaling and the TGF-β pathway, both of which are essential for the proliferation and differentiation of stem cells into tendon-like cells [50]. Our findings demonstrate that TGFBR2 is a downstream target of miR-337 and CRNDE, with both its mRNA and protein levels modulated by the CRNDE/miR-337 axis. Furthermore, overexpression of CRNDE was able to restore tenogenic differentiation by regulating TGFBR2 expression, both *in vitro* and *in vivo*.

In summary, this study reveals that the lncRNA CRNDE enhances tenogenic differentiation of TDSCs and promotes tendon-bone healing through the regulation of the miR-337/TGFBR2 signaling axis. These findings offer new insights into the molecular mechanisms involved in tendon repair and highlight CRNDE as a promising therapeutic target for rotator cuff injuries.

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Disclosure of conflict of interest

None.

Abbreviations

AA, ascorbic acid; CRNDE, colorectal neoplasia differentially expressed; COL1A1, collagen type I; DCN, decorin; ECM, extracellular matrix; FBS, fetal bovine serum; FMOD, fibromodulin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HEK, human embryonic kidney; lncRNA, long noncoding RNA; MKX, Mohawk; MSC, mesenchymal stem cell; qPCR, quantitative PCR; RCT, rotator cuff tear; SCX, scleraxis; TGFBR2, transforming growth factor beta receptor 2; TDSC, tendon-derived stem cell; TSPCs, human tendon stem/progenitor cells; TNMD, tenomodulin.

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