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Original Article

Effects of ADRM1 on osteoblast differentiation and mineralization in osteoporosis

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Abstract: Objective: Adhesion regulating molecule-1 (ADRM1), a 26S proteasome adaptor protein, plays a crucial role in mediating the growth and differentiation of osteoclasts in osteoporosis (OP). However, its involvement in this osteoanabolic effect remains largely uninvestigated. Methods: *In vitro* experiments, including both gain-of-function and loss-of-function approaches, were conducted in MC3T3-E1 and C3H10T1/2 cells. Results: Knockdown of ADRM1 markedly promoted the growth of C3H10T1/2 cells while inhibiting apoptosis. Additionally, this intervention enhanced the expression of osteoblast differentiation markers and key proteins associated with the Wnt/β-catenin pathway. Notably, silencing ADRM1 promoted osteoblast mineralization and differentiation, as evidenced by increased Alizarin red staining and alkaline phosphatase staining. Conversely, MC3T3-E1 cells overexpressing ADRM1 exhibited results that were diametrically opposed to those observed with ADRM1 knockdown. Furthermore, treatment with ICG-001 (a Wnt/β-catenin pathway antagonist) reversed the effects of ADRM1 knockdown in C3H10T1/2 cells. Conclusions: Our findings suggest that silencing ADRM1 induces osteoblast mineralization and differentiation by activating the Wnt/β-catenin pathway. This finding underscores the therapeutic potential of the ADRM1/Wnt/β-catenin axis in treating OP.

Keywords: Osteoporosis, ADRM1, osteoblast, differentiation, mineralization

Introduction

Osteoporosis (OP) is a prevalent systemic metabolic bone disease characterized by decreased bone mass, altered bone microstructure, reduced bone strength, increased bone fragility, and an elevated risk of fractures [1]. The pathogenesis of OP involves a disruption in the dynamic balance of bone remodeling, leading to an imbalance in the functions of osteoblasts and osteoclasts. This results in impaired bone metabolism and a net loss of bone volume [2]. Current pharmacological interventions, such as bisphosphonates and calcitonin, which are widely utilized in clinical practice, primarily slow OP progression by inhibiting bone resorption. However, these treatments do not significantly enhance the microstructural integrity of trabecular bones [3]. Consequently, enhancing bone formation has become a central focus of OP treatment.

The 26S proteasome plays a pivotal role in the ubiquitin-proteasome pathway, accounting for over 80% of protein degradation within mammalian cells [4]. Cancer cells exploit this pathway to trigger continuous pro-tumor signaling cascades, thereby facilitating cell cycle progression and inhibiting cell death caused by abnormal stress [5]. Adhesion regulating molecule-1 (ADRM1), identified as a 26S proteasome adaptor protein, is overexpressed in various solid tumors and plays a significant role in tumorigenesis [6-8]. Recently, exploration of crucial functions of the ubiquitin proteasome pathway in skeletal cell differentiation and bone homeostasis has gained attention. Dysregulated ubiquitination can disrupt bone

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cell differentiation, affecting bone homeostasis and ultimately leading to OP occurrence [9]. Chandra et al. revealed that bortezomib, a proteasome inhibitor, enhances both the number and activity of osteoblasts in a mouse model of OP while reducing osteoclast quantity and function, contributing to the reversal of trabecular bone structure and strength loss [10]. Additionally, Kim et al. showed that knockdown of ADRM1 significantly inhibits osteoclast differentiation and proliferation [11]. However, the involvement and mechanism by which ADRM1 participates in osteoblast differentiation are still at an early stage.

In this study, we conducted a preliminary investigation into the role and mechanism of ADRM1 in osteoblast differentiation and mineralization. Through gain-of-function and loss-of-function experiments, we elucidated the function of the ADRM1/Wnt/β-catenin axis and verified our findings at the cellular level. This work further reveals the mechanisms by which ADRM1 affects the osteogenic differentiation in OP.

Methods

Cell culture and transfection

Mouse pluripotent mesenchymal stem cell-like fibroblasts C3H10T1/2 and mouse calvarial preosteoblasts MC3T3-E1 were obtained from Pricella Biotechnology (Wuhan, China). The cells were incubated in either MEM or DMEM (Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (Gibco, Rockville, MD, USA) and 1% streptomycin/penicillin. For osteoblast differentiation induction, MC3T3-E1 cells were incubated in complete medium containing 5 mM β-glycerophosphate and 25 μg/ml ascorbic acid, while C3H10T1/2 cells were incubated in complete medium containing 5 mM β-glycerophosphate, 25 μg/ml ascorbic acid, and 10 nM dexamethasone.

ADRM1 overexpression (OE-ADRM1), ADRM1 silencing (si-ADRM1-1/-2), and corresponding negative controls (OE-NC and si-NC) were synthesized by General Biol (Chuzhou, China). Following the guidelines for Lipofectamine 3000 transfection reagent (Invitrogen, Karlsruhe, Germany), C3H10T1/2 cells were co-cultured with either si-NC or si-ADRM1-1/-2, while MC3T3-E1 cells were incubated with OE-NC or OE-ADRM1 for 48 h. Transfected cells were then used for subsequent *in vitro* experiments.

Osteoblast differentiation and mineralization assays

According to the user manual, the alkaline phosphatase (ALP) activity and calcium deposits in C3H10T1/2 and MC3T3-E1 cells were assessed using an ALP assay Kit (Beyotime, Beijing, China) and Alizarin red staining (ARS) Kit (Cyagen Biosciences, Guangzhou, China), respectively. ALP staining was performed 5 days after culture, while ARS was conducted after 14 days of culture, since extracellular matrix (ECM) mineralization represents the terminal step of osteoblast differentiation.

Cell growth potential measurement

Growth potential in MC3T3-E1 and C3H10T1/2 cells was evaluated using a Cell Counting Kit-8 (CCK-8) assay. The cells (3×10^3 cells/mL) were cultured in 96-well plates for different durations (0, 24, 48, and 72 h). Then, the CCK-8 solution (15 μL; Dojindo Laboratories, Shanghai, China) was added, and cell viability was subsequently measured using a microplate reader (Thermo Fisher Scientific, Rockford, MD, USA) after a 2-hour incubation.

Fow cytometer

For cell cycle analysis, a total of 1×10^5 cells were initially treated with 75% ethanol for 1 h. Following centrifugation for 5 min at 1500 g, the cells were resuspended and incubated with RNase A/propidium iodide solution (Vazyme, Nanjing, China) for 30 min in the dark. The distribution of cells across the G1, S, and G2 phases was assessed using a FACScan flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Additionally, the apoptotic rate of cells was analyzed by incubating the resuspended cells with propidium iodide/Annexin V-FITC for 20 min in the dark.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Trizol (Invitrogen) was employed for the extraction of total RNA, followed by quantification

Table 1. Real-time PCR primer synthesis list

Gene	Sequences	
Cnx43	Forward	5'-TCTGTCCCACCTTTGTGTC-3'
	Reverse	5'-CTTGCCTCCCTGATGCT-3'
Axin2	Forward	5'-CGAGTGACGAATTTGCCT-3'
	Reverse	5'-CGATCCTCTCCACTTTGC-3'
Lef1	Forward	5'-TATGAACAGCGACCCGTA-3'
	Reverse	5'-CGGAGAAAAGTGCTCGTC-3'
ADRM1	Forward	5'-AAGTACTTGGTGGAGTTTCG-3'
	Reverse	5'-ATGATCAAGTCGTCTTCCAC-3'
GAPDH	Forward	5'-CCACCCATGGCAAATTCCATGGCA-3'
	Reverse	5'-TCTAGACGGCAGGTCAGGTCCACC-3'

Cnx43, Connexin 43; Axin2, Axis inhibition protein 2; Lef1, Lymphoid enhancer binding factor 1; ADRM1, Adhesion regulating molecule-1.

using a Nanodrop2000 (Thermo Fisher Scientific). cDNA synthesis was performed according to the PrimeScript RT reagent Kit (Invitrogen) instructions. qRT-PCR assays were conducted with SYBR Green Premix (Invitrogen) on a QuantStudio7 machine (Thermo Fisher Scientific). The relative expression of target genes was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. **Table 1** lists the primers used for qRT-PCR.

Western blotting

Protein extracts were separated by 10% polyacrylamide gel electrophoresis and then electro-transferred onto PVDF membranes by electroblotting. Prior to the incubation of primary antibodies (all dilution in 1:1000), the membrane was blocked using 5% nonfat milk. Afterward, the membrane was incubated with the corresponding secondary antibodies (Abcam; 1:10000) for 1 h. Protein bands were visualized using an ECL kit (Beyotime) and an imaging system (Bio-Rad, Hercules, CA, USA). **Table 2** lists the sources of primary antibodies.

Statistical analysis

Data analysis was performed using SPSS 22.0. Results were expressed as mean ± standard deviation. Statistical differences between two groups were evaluated utilizing Student's t-test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A significance threshold of $P < 0.05$ was considered significant.

Results

Effects of ADRM1 overexpression or silencing on the proliferation and apoptosis of osteoblasts

The expression of ADRM1 in MC-3T3-E1 and C3H10T1/2 cells was determined. As shown in **Figure 1A**, ADRM1 expression was significantly reduced in MC3T3-E1 cells compared to that of C3H10T1/2 cells ($P < 0.001$). To investigate the specific role of ADRM1 in primary OP, MC3T3-E1 cells were transfected with OE-ADRM1 plasmid to achieve stable overexpression of ADRM1, while C3H10T1/2 underwent si-ADRM1-1/-2 transfection to achieve ADRM1 interference. The results demonstrated that ADRM1 was markedly overexpressed in MC3T3-E1 cells ($P < 0.001$, **Figure 1B**), but at low levels in C3H10T1/2 cells, particularly in si-ADRM1-1 transfected C3H10T1/2 cells ($P < 0.001$, **Figure 1C**).

MC3T3-E1 cells transfected with OE-ADRM1 showed a marked decrease in cell viability ($P < 0.01$, **Figure 1D**), while C3H10T1/2 cell viability was dramatically increased when ADRM1 was silenced ($P < 0.01$, **Figure 1E**). Since cell viability is strongly linked to the cell cycle, we further analyzed the cell cycle distribution in both cell lines. In comparison to the OE-NC group, MC3T3-E1 cells overexpressing ADRM1 exhibited a significant increase in the proportion of cells in the G1 phase ($P < 0.01$) and a decrease in the S phase ($P < 0.001$) (**Figure 1F**). On the contrary, silencing ADRM1 in C3H10T1/2 cells resulted in a significant reduction in the G1 phase ($P < 0.01$) and an increase in the S phase ($P < 0.001$) (**Figure 1G**).

As illustrated in **Figure 1H**, high ADRM1 expression promoted apoptosis in MC3T3-E1 cells ($P < 0.001$), while silencing ADRM1-1 in C3H10T1/2 cells distinctly suppressed apoptosis ($P < 0.001$, **Figure 1I**). Western blotting further validated these findings by showing that overexpression of ADRM1 in MC3T3-E1 cells significantly increased the levels of pro-apoptotic proteins Bax, Cleaved Caspase 3, and Cleaved Caspase 9, but decreased the levels of cell cycle-related proteins Cyclin A1, Cyclin

Table 2. Primary antibodies for western blotting

Primary antibody	Catalog number	Dilution ratio	Source
Bax	ab32503	1:1,000	Abcam, Cambridge, UK
Cleaved Caspase 3	ab214430	1:1,000	Abcam, Cambridge, UK
Cleaved Caspase 9	#9509	1:1,000	Cell Signaling Technology, Boston, MA, USA
Cyclin A1	13295-1-AP	1:500	Proteintech, Wuhan, China
Cyclin B1	67686-1-Ig	1:1,000	Proteintech, Wuhan, China
Cyclin D2	67048-1-Ig	1:1,000	Proteintech, Wuhan, China
Runx2	ab236639	1:1,000	Abcam, Cambridge, UK
Collagen I	67288-1-Ig	1:1,000	Proteintech, Wuhan, China
Osterix	ab209484	1:1,000	Abcam, Cambridge, UK
Osteocalcin	ab309521	1:2,000	Abcam, Cambridge, UK
Osteopontin	ab218237	1:5,000	Abcam, Cambridge, UK
p-GSK-3β (Ser9)	#9336	1:2,000	Cell Signaling Technology, Boston, MA, USA
GSK-3β	GSK-3β	1:5,000	Cell Signaling Technology, Boston, MA, USA
β-catenin	#9562	1:2,000	Cell Signaling Technology, Boston, MA, USA
GAPDH	60004-1-Ig	1:50,000	Proteintech, Wuhan, China
Histone H3	17168-1-AP	1:50,000	Proteintech, Wuhan, China

Runx2, Runt-related transcription factor 2; Osx, Osterix; Ocn, Osteocalcin; OPN, Osteopontin.

B1, and Cyclin D2 ($P < 0.01$, **Figure 1J**). In contrast, C3H10T1/2 cells transfected with si-ADRM1-1 exhibited the opposite pattern of protein expression ($P < 0.01$, **Figure 1K**).

Effects of OE-ADRM1 and si-ADRM1-1 on osteoblast differentiation and mineralization

The influences of ADRM1 overexpression and silencing on the differentiation and mineralization of osteoblasts were subsequently assessed. Initially, ALP activity, a crucial and widely recognized marker for early osteoblast differentiation, was assessed. Following this, ARS staining was done to characterize mineralization, as the final stage of osteoblast differentiation involves the formation of ECM mineralization.

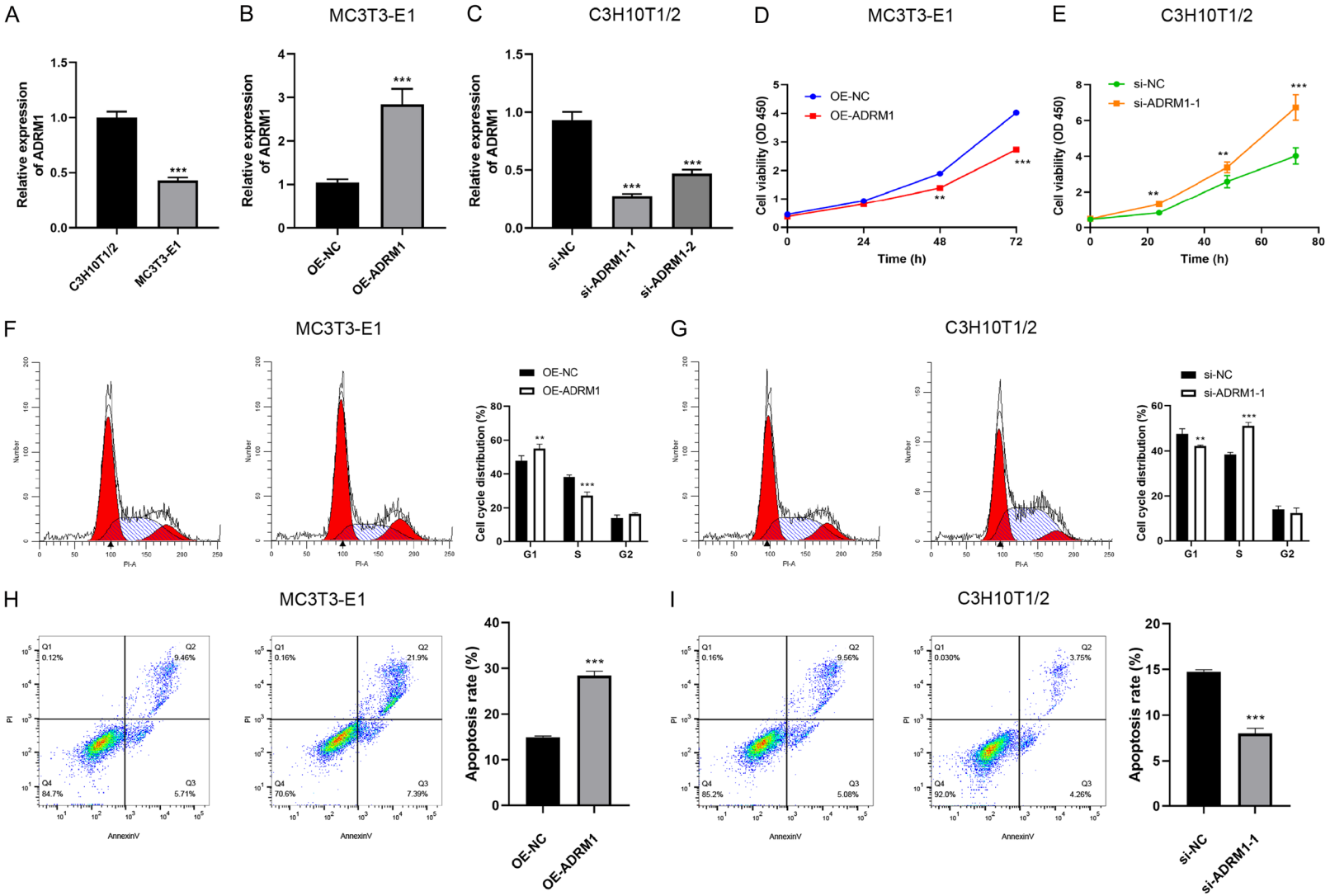
As illustrated in **Figure 2A**, ALP activity was markedly inhibited in MC3T3-E1 cells transfected with OE-ADRM1 ($P < 0.01$). Furthermore, ARS staining revealed a reduction in the number of calcified ECM plaques after OE-ADRM1 transfection ($P < 0.01$). In contrast, si-ADRM1-1 resulted in the opposite results ($P < 0.001$, **Figure 2B**).

Moreover, the protein levels of osteoblast differentiation markers, including runt-related transcription factor 2 (Runx2), osterix (Osx), osteocalcin (Ocn), collagen I and osteopontin (OPN), were markedly downregulated upon OE-ADRM1 transfection in MC3T3-E1 cells ($P < 0.01$, **Figure 2C**) but upregulated upon transfection with si-ADRM1-1 in C3H10T1/2 cells ($P < 0.05$, **Figure 2D**).

Effects of OE-ADRM1 and si-ADRM1-1 on Wnt/β-catenin pathway

The Wnt/β-catenin signaling pathway plays a critical role in osteoblast differentiation [12]. To verify whether ADRM1 modulates this pathway, we evaluated the expression of β-catenin, a key component the Wnt/β-catenin pathway, following transfection with OE-ADRM1 or si-ADRM1-1.

Figure 3A shows that total β-catenin levels were markedly reduced in MC3T3-E1 cells upon OE-ADRM1 transfection ($P < 0.01$). Moreover, the nuclear β-catenin expression was also downregulated ($P < 0.01$). The phosphorylation of GSK-3β at the Ser9 site is essential for initiating the Wnt/β-catenin pathway [13]. We observed that OE-ADRM1 transfection significantly inhibited the phosphorylation of GSK-3β at Ser9 ($P < 0.01$, **Figure 3A**), while the total GSK-3β expression remained unchanged. Conversely, in C3H10T1/2 cells transfected with si-ADRM1-1, the expression of total β-catenin, nuclear β-catenin and p-GSK-3β (Ser9) was significantly increased ($P < 0.01$,


A
Relative expression of ADRM1
C3H10T1/2
MC3T3-E1
B
MC3T3-E1
Relative expression of ADRM1
OE-NC
OE-ADRM1
C
C3H10T1/2
Relative expression of ADRM1
si-NC
si-ADRM1-1
si-ADRM1-2
D
MC3T3-E1
Cell viability (OD 450)
OE-NC
OE-ADRM1
Time (h)
E
C3H10T1/2
Cell viability (OD 450)
si-NC
si-ADRM1-1
Time (h)
F
MC3T3-E1
Number
PI-A
Cell cycle distribution (%)
OE-NC
OE-ADRM1
G1
S
G2
G
C3H10T1/2
si-NC
si-ADRM1-1
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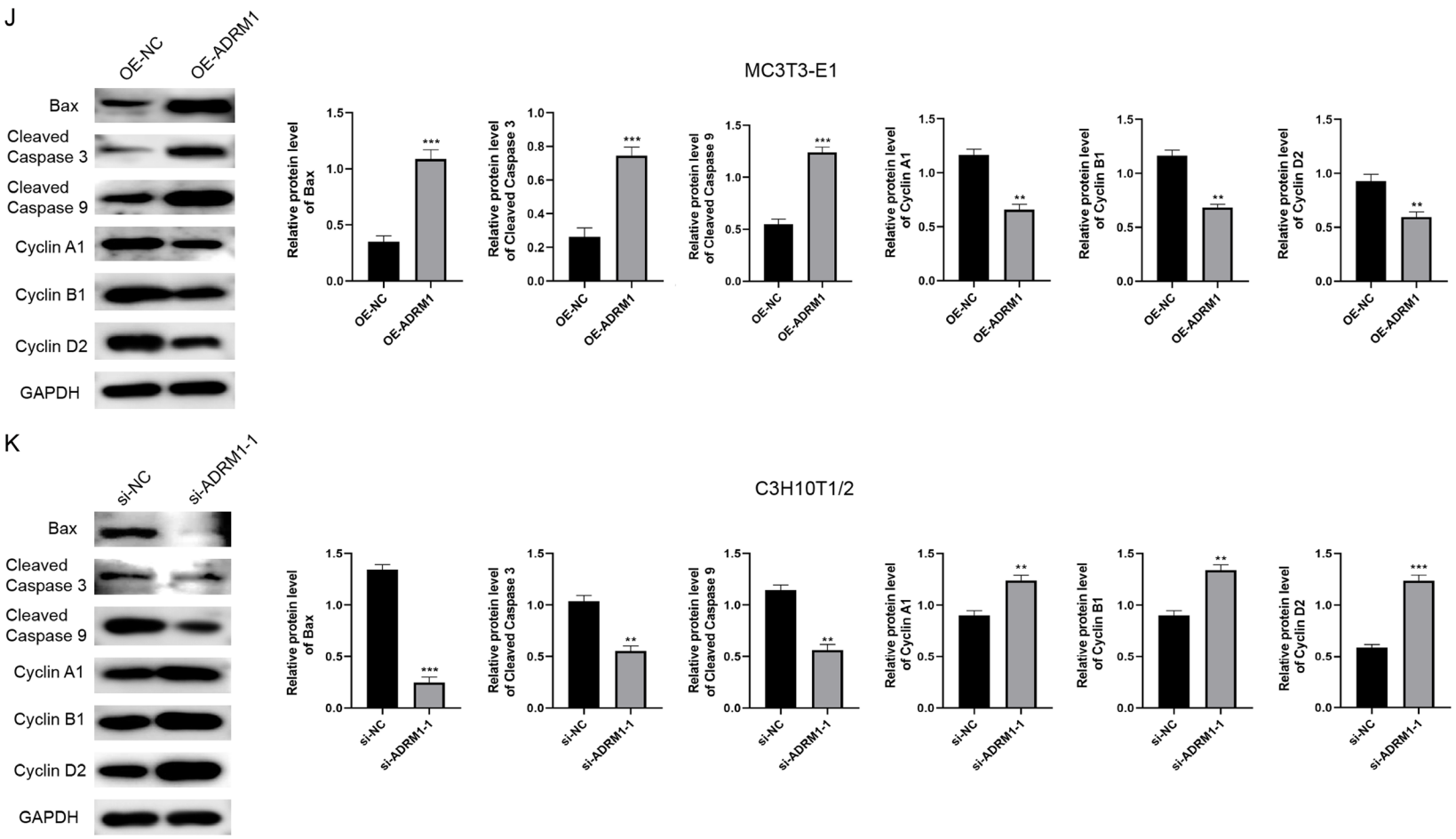
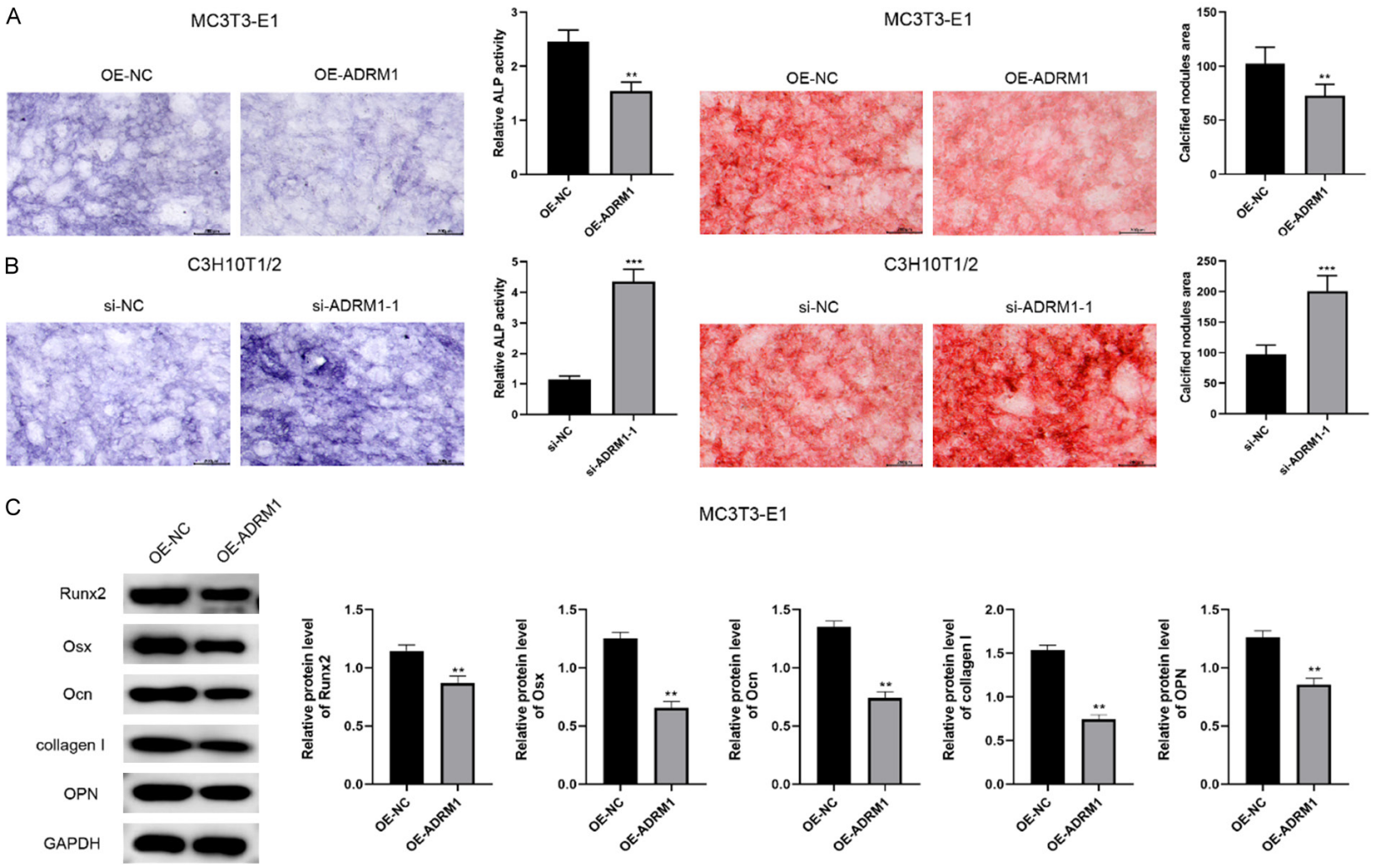


Figure 1. Effects of adhesion regulating molecule-1 (ADRM1) overexpression or silencing on the proliferation and apoptosis of osteoblasts. (A) The expression of ADRM1 in MC3T3-E1 and C3H10T1/2 cells was determined through qRT-PCR. The expression of ADRM1 in (B) MC3T3-E1 cells transfected with OE-ADRM1 or in (C) C3H10T1/2 cells transfected with si-ADRM1-1/-2 was quantified by qRT-PCR. The viability of (D) MC3T3-E1 cells or (E) C3H10T1/2 cells was measured by CCK-8 assay. Following transfection of OE-ADRM1 or si-ADRM1-1, the cell cycle of (F) MC3T3-E1 cells or (G) C3H10T1/2 cells was analyzed by flow cytometry. Flow cytometry was used to analyze the apoptosis rate of (H) MC3T3-E1 cells or (I) C3H10T1/2 cells. The levels of proapoptotic- and cell cycle-related proteins in (J) MC3T3-E1 cells or (K) C3H10T1/2 cells were measured by western blotting. $^{**}P < 0.01$, $^{***}P < 0.001$. *In vitro* experiments were performed in triplicate, and each experiment was repeated 3 times.


A
MC3T3-E1
OE-NC
OE-ADRM1
Relative ALP activity
MC3T3-E1
OE-NC
OE-ADRM1
Calcified nodules area
B
C3H10T1/2
si-NC
si-ADRM1-1
Relative ALP activity
C3H10T1/2
si-NC
si-ADRM1-1
Calcified nodules area
C
OE-NC
OE-ADRM1
Runx2
Osx
Ocn
collagen I
OPN
GAPDH
MC3T3-E1
Relative protein level of Runx2
Relative protein level of Osx
Relative protein level of Ocn
Relative protein level of collagen I
Relative protein level of OPN

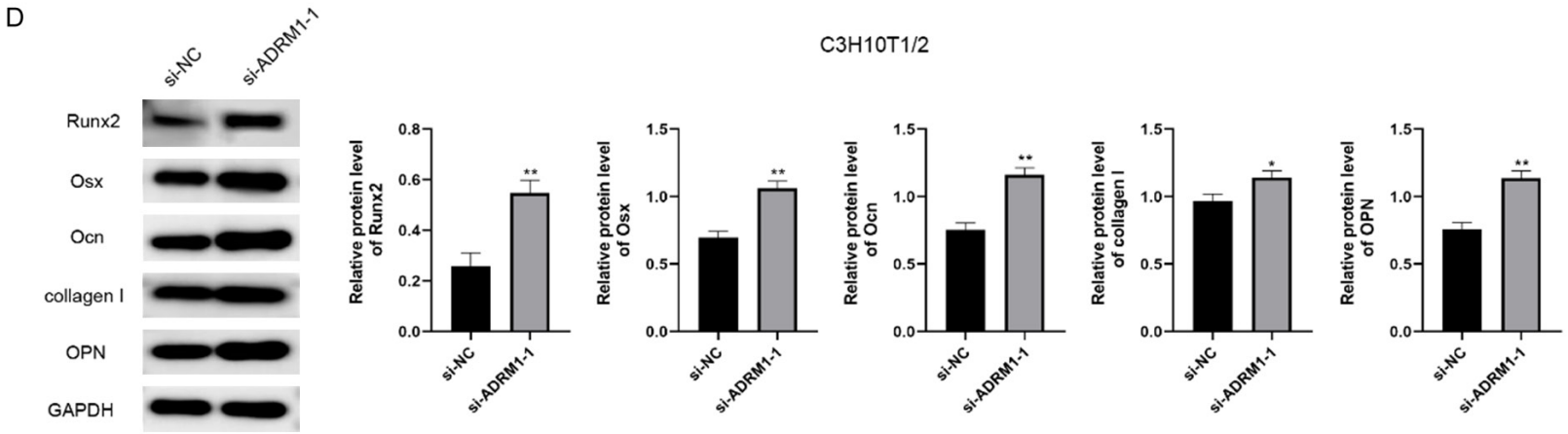
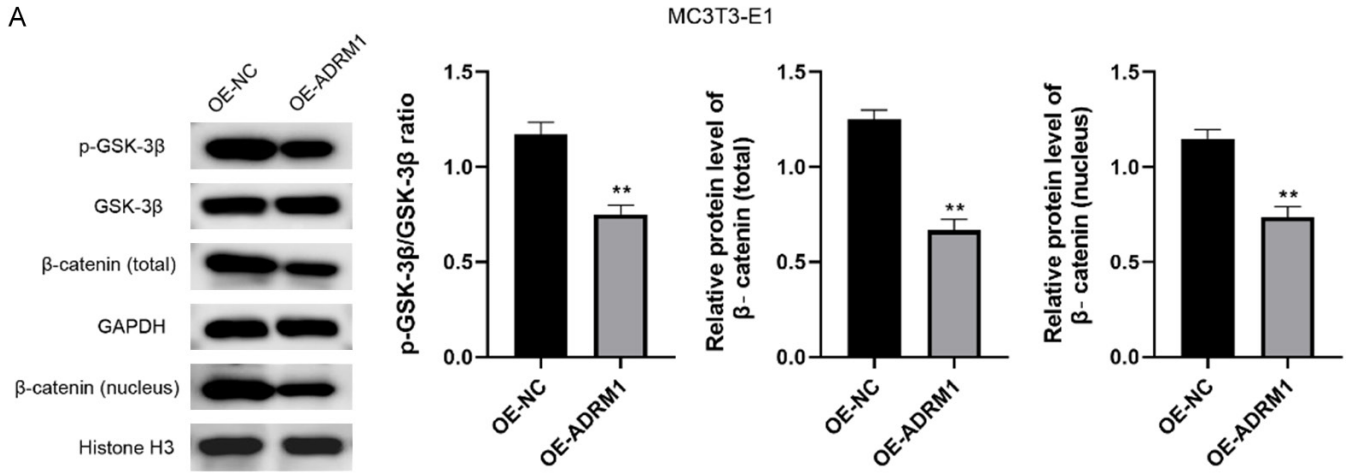


Figure 2. The effect of OE-adhesion regulating molecule-1 (ADRM1) and si-ADRM1-1 on osteoblast differentiation and mineralization. Alkaline phosphatase (ALP) activity and calcified nodules area in (A) MC3T3-E1 cells transfected with OE-ADRM1 or in (B) C3H10T1/2 cells transfected with si-ADRM1-1. The levels of osteoblast differentiation markers in (C) MC3T3-E1 cells transfected with OE-ADRM1 or in (D) C3H10T1/2 cells transfected with si-ADRM1-1 were measured by western blotting. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *In vitro* experiments were performed in triplicate, and each experiment was repeated 3 times.



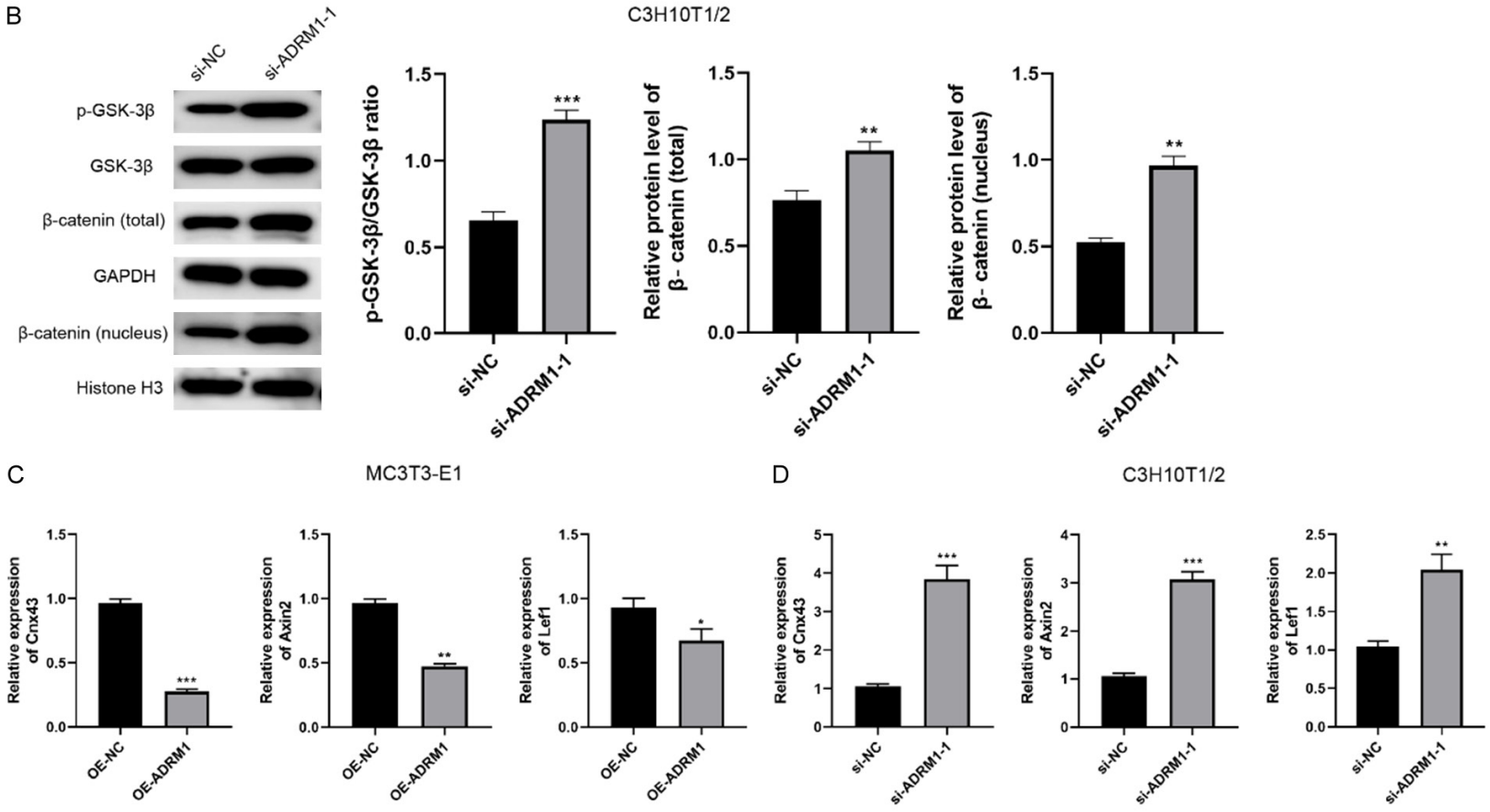


Figure 3. The effect of OE-adhesion regulating molecule-1 (ADRM1) and si-ADRM1-1 on Wnt/β-catenin pathway. The levels of key proteins in Wnt/β-catenin pathway in (A) MC3T3-E1 cells transfected with OE-ADRM1 or in (B) C3H10T1/2 cells transfected with si-ADRM1-1 were measured by western blotting. The mRNA expression levels of downstream genes in the Wnt/β-catenin pathway were subsequently analyzed in both (C) MC3T3-E1 and (D) C3H10T1/2 cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *In vitro* experiments were performed in triplicate, and each experiment was repeated 3 times.

Figure 3B). Subsequently, the mRNA expression levels of downstream genes in the Wnt/β-catenin pathway were analyzed in both MC3T3-E1 and C3H10T1/2 cells. Our results indicated that the mRNA expression of Cnx43, Axin2, and Lef1 were significantly suppressed upon ADRM1 overexpression ($P < 0.05$, **Figure 3C**), whereas silencing ADRM1 led to an increase in their expressions ($P < 0.01$, **Figure 3D**).

Silencing of ADRM1 modulated osteoblast differentiation and mineralization via Wnt/β-catenin pathway

A specific antagonist of Wnt/β-catenin pathway [14], ICG-001, was introduced into C3H-10T1/2 cells to investigate the interplay of ADRM1 with Wnt/β-catenin pathway. Our results showed that ICG-001 (5 μM) effectively counteracted the enhancing effects of ADRM1 knockdown on the levels of total β-catenin protein, nuclear β-catenin, and p-GSK-3β (Ser9) ($P < 0.05$, **Figure 4A**). Furthermore, the increase in ALP activity and ECM mineralization resulting from ADRM1 silencing was also partially weakened following ICG-001 treatment ($P < 0.01$, **Figure 4B**). Additionally, we assessed the protein levels of osteoblast differentiation markers. As shown in **Figure 4C**, the elevation in Runx2, Osx, Ocn, collagen I, and OPN proteins induced by ADRM1 silencing was markedly inhibited upon ICG-001 treatment ($P < 0.05$).

Discussion

Bone tissue is in a constant state of remodeling, where osteoblasts and osteoclasts work together to maintain bone homeostasis. Under normal physiologic conditions, osteoblasts are responsible for bone formation, while osteoclasts facilitate bone resorption. In osteoporosis (OP), however, an imbalance occurs when bone resorption by osteoclasts exceeds the rate of bone formation by osteoblasts [15, 16]. The primary therapeutic goal in treating OP is to enhance bone strength and density, which can help reduce the risk of fractures. This can be achieved through strategies that either minimize bone resorption or stimulate bone formation. In this context, our research focuses on understanding the role of ADRM1 in modulating osteoblast differentiation and mineralization through its interaction with the Wnt/β-catenin signaling pathway.

ADRM1 functions as an adaptor protein for the 26S proteasome, facilitating a connection between the 26S proteasome and its substrates, contributing to the regulation of various cellular processes through protein degradation. Studies have suggested that ADRM1 may also have significant implications in cancer biology. For example, Yu et al. found that bladder elevated levels of ADRM1 in cancer patients were associated with dismal survival outcomes [7], while Liang et al. reported that silencing ADRM1 can could significantly inhibit hepatocellular carcinoma cell growth [17]. Furthermore, given the increasing recognition of the ubiquitin-proteasome pathway in regulating cell differentiation and maintaining bone homeostasis, we speculated that ADRM1 may play a critical role in mediating both the growth and differentiation of osteoblasts in the context of OP. In our study, we demonstrated that ADRM1 overexpression suppressed the proliferative potential and induced apoptosis of MC3T3-E1 cells, while silencing of ADRM1 in C3H10T1/2 cells led to increased cell viability and proliferation. Moreover, we assessed the levels of markers for osteoblast differentiation at various stages. Pre-osteoblasts are often characterized by specific expression of transcription factors such as Runx2 and Osx, while OPN is upregulated during the middle stages of differentiation, and Ocn is a characteristic marker of mature osteoblasts [18]. Collagen I, a key extracellular matrix component, is expressed throughout the differentiation process [19]. We found that overexpression of ADRM1 suppressed the protein levels of Runx2, Osx, Ocn, collagen I and OPN, while silencing ADRM1 promoted their expression. To further assess osteoblast differentiation and mineralization, we conducted ALP staining and ARS. Both assays revealed that silencing ADRM1 in C3H10T1/2 cells promoted both the early and late stages of osteoblast differentiation and mineralization.

The differentiation of osteoblasts is primarily regulated by the Wnt/β-catenin pathway, which acts as a key modulator of osteogenesis in conjunction with bone morphogenetic proteins [20, 21]. This pathway also significantly influences the fate of mesenchymal stem cells. In

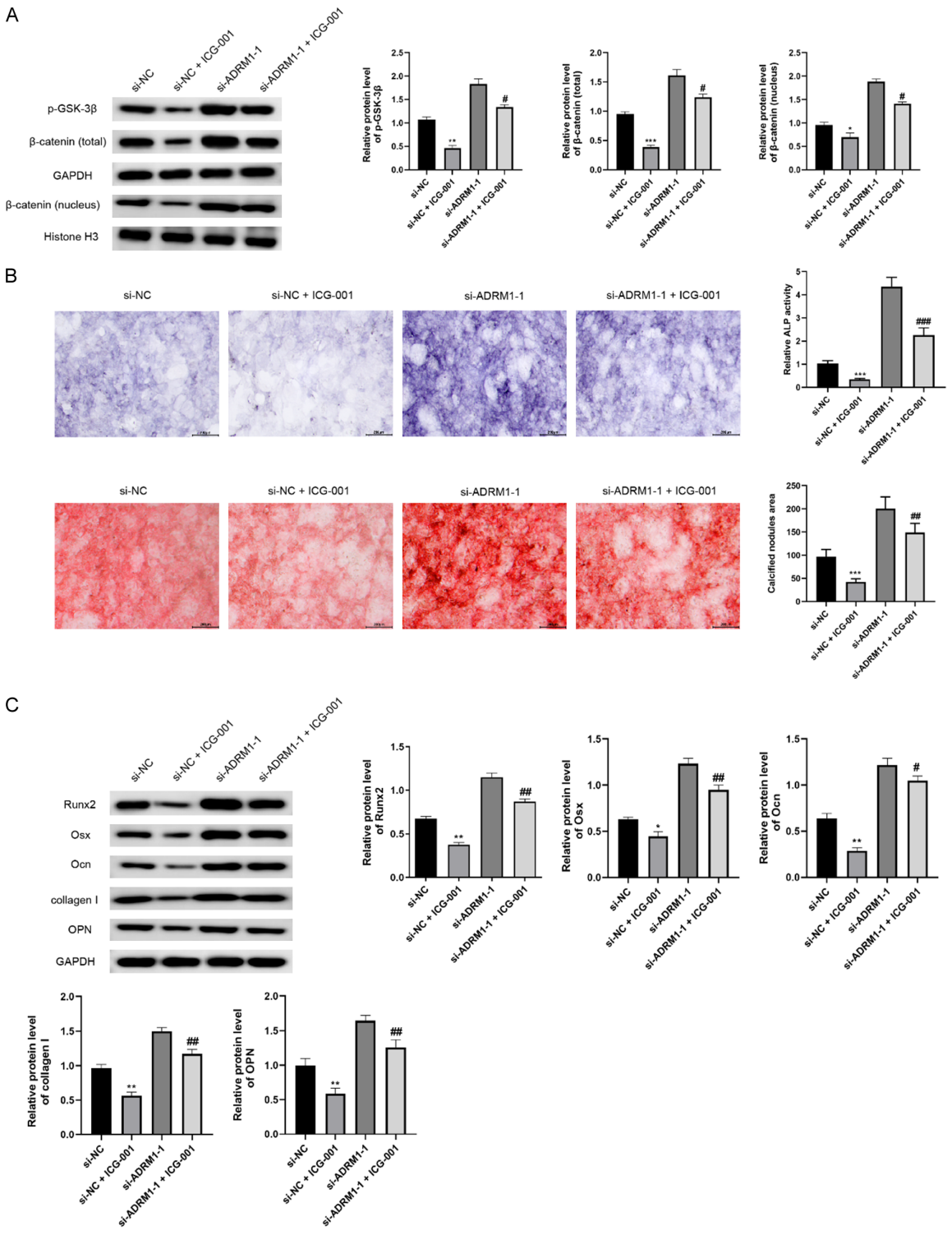


Figure 4. Silencing of adhesion regulating molecule-1 (ADRM1) modulated osteoblast differentiation and mineralization through the Wnt/β-catenin pathway. A. Following treatment with ICG-001, the levels of important proteins in Wnt/β-catenin pathway in C3H10T1/2 cells transfected with si-ADRM1-1 were measured by western blotting. B. Following treatment with ICG-001, ALP activity and calcified nodules area in C3H10T1/2 cells were determined. C. Following treatment with ICG-001, the levels of osteoblast differentiation markers in C3H10T1/2 cells were measured by western blotting. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to si-NC group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to si-ADRM-1 group. *In vitro* experiments were performed in triplicate, and each experiment was repeated 3 times.

the absence of β-catenin, mesenchymal stem cells fail to differentiate into fully mature osteoblasts, characterized by the expression of Ocn; instead, they preferentially differentiate into chondrocytes [22-24]. Central to the initiation of this pathway is the phosphorylation of GSK-3β at Ser9 and the subsequent nuclear translocation of β-catenin, which together drive the activation of Wnt/β-catenin signaling [13, 25]. A variety of compounds, including metformin, bergamottin and T63, have been reported to activate the Wnt/β-catenin pathway and mitigate the progression of OP [26-28]. Given the osteoanabolic role of ADRM1 in C3H10T1/2 cells, we hypothesized that ADRM1 knockdown may activate the Wnt/β-catenin pathway, further promoting osteoblast differentiation and mineralization. As expected, we verified that the suppression of ADRM1 not only increased p-GSK-3β (Ser9) levels, but also facilitated the nuclear accumulation of β-catenin and enhanced the expression of downstream genes within the Wnt/β-catenin pathway. Furthermore, we utilized ICG-001 to inhibit this pathway and subsequently investigated its reciprocal interaction with ADRM1. Our results demonstrated that introducing ICG-001 into C3H10T1/2 cells led to a reverse of promoting effects of ADRM1 knockdown on osteoblast differentiation and mineralization, as evidenced by decreased ALP activities, a reduction in the area of calcified nodules, and diminished expression of osteoblast differentiation markers and key Wnt/β-catenin pathway proteins.

Conclusion

Collectively, the present study preliminarily elucidates the role of ADRM1 in the progression of OP *in vitro*. It highlights that ADRM1 knockdown promotes osteoblast differentiation and mineralization through the activation of the Wnt/β-catenin pathway. These results suggest the therapeutic potential of targeting the ADRM1/Wnt/β-catenin axis as a strategy for combating OP and enhancing bone regeneration.

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Disclosure of conflict of interest

None.

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References

[1] Gopinath V. Osteoporosis. Med Clin North Am 2023; 107: 213-225.
[2] Adejuyigbe B, Kallini J, Chiou D and Kallini JR. Osteoporosis: molecular pathology, diagnostics, and therapeutics. Int J Mol Sci 2023; 24: 14583.
[3] Aibar-Almazan A, Voltes-Martinez A, Castellote-Caballero Y, Afanador-Restrepo DF, Carcelen-Fraile MDC and Lopez-Ruiz E. Current status of the diagnosis and management of osteoporosis. Int J Mol Sci 2022; 23: 9465.
[4] Collins GA and Goldberg AL. The logic of the 26S proteasome. Cell 2017; 169: 792-806.
[5] Bedford L, Lowe J, Dick LR, Mayer RJ and Brownell JE. Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. Nat Rev Drug Discov 2011; 10: 29-46.
[6] Shao Z, Wang Y, He Y, Zhang C, Zhao Y, Zhang M, Li Q and Wang J. The high expression of ADRM1 in hepatocellular carcinoma is closely related to tumor immune infiltration and is regulated by miR-891a-5p. Sci Rep 2024; 14: 14002.
[7] Yu QX, Wang JC, Liu JF, Ye LX, Guo YQ and Zheng HH. Adhesion-regulating molecule 1 (ADRM1) can be a potential biomarker and target for bladder cancer. Sci Rep 2023; 13: 14803.
[8] Wang J, Wu X, Dai W, Li J, Xiang L, Tang W, Lin J, Zhang W, Liu G, Yang Q, Lin Z, Sun Y, Zhang Y, Chen Y, Li G, Li A, Liu S, Li Y and Wang J. The CCDC43-ADRM1 axis regulated by YY1, promotes proliferation and metastasis of gastric cancer. Cancer Lett 2020; 482: 90-101.
[9] Fan X, Zhang R, Xu G, Fan P, Luo W, Cai C and Ge RL. Role of ubiquitination in the occurrence and development of osteoporosis (Review). Int J Mol Med 2024; 54: 68.
[10] Chandra A, Wang L, Young T, Zhong L, Tseng WJ, Levine MA, Cengel K, Liu XS, Zhang Y, Pignolo RJ and Qin L. Proteasome inhibitor bortezomib is a novel therapeutic agent for focal radiation-induced osteoporosis. FASEB J 2018; 32: 52-62.
[11] Kim T, Ha HI, Kim N, Yi O, Lee SH and Choi Y. Adrm1 interacts with Atp6v0d2 and regulates osteoclast differentiation. Biochem Biophys Res Commun 2009; 390: 585-590.

[12] Vlashi R, Zhang X, Wu M and Chen G. Wnt signaling: essential roles in osteoblast differentiation, bone metabolism and therapeutic implications for bone and skeletal disorders. Genes Dis 2023; 10: 1291-1317.
[13] Gao C, Chen G, Romero G, Moschos S, Xu X and Hu J. Induction of Gsk3beta-beta-TrCP interaction is required for late phase stabilization of beta-catenin in canonical Wnt signaling. J Biol Chem 2014; 289: 7099-7108.
[14] Akcora BO, Storm G and Bansal R. Inhibition of canonical WNT signaling pathway by beta-catenin/CBP inhibitor ICG-001 ameliorates liver fibrosis in vivo through suppression of stromal CXCL12. Biochim Biophys Acta Mol Basis Dis 2018; 1864: 804-818.
[15] Xu J, Yu L, Liu F, Wan L and Deng Z. The effect of cytokines on osteoblasts and osteoclasts in bone remodeling in osteoporosis: a review. Front Immunol 2023; 14: 1222129.
[16] Kaur M, Nagpal M and Singh M. Osteoblast-n-osteoclast: making headway to osteoporosis treatment. Curr Drug Targets 2020; 21: 1640-1651.
[17] Liang YC, Wang JL, Wang HT, Liu H, Zhang HL and Liang YX. ADRM1 as a therapeutic target in hepatocellular carcinoma. Kaohsiung J Med Sci 2021; 37: 47-54.
[18] Amarasekara DS, Kim S and Rho J. Regulation of osteoblast differentiation by cytokine networks. Int J Mol Sci 2021; 22: 2851.
[19] Alajmi K, Hartford M, Roy NS, Bhattacharya A, Kaity S, Cavanagh BL, Roy S and Kaur K. Selenium nanoparticle-functionalized injectable chitosan/collagen hydrogels as a novel therapeutic strategy to enhance stem cell osteoblastic differentiation for bone regeneration. J Mater Chem B 2024; 12: 9268-9282.
[20] Gao Y, Chen N, Fu Z and Zhang Q. Progress of Wnt signaling pathway in osteoporosis. Biomolecules 2023; 13: 483.
[21] Marini F, Giusti F, Palmini G and Brandi ML. Role of Wnt signaling and sclerostin in bone and as therapeutic targets in skeletal disorders. Osteoporos Int 2023; 34: 213-238.
[22] Wong SK, Mohamad NV, Jayusman PA and Ibrahim N. A review on the crosstalk between insulin and Wnt/beta-catenin signalling for bone health. Int J Mol Sci 2023; 24: 12441.
[23] Lin W, Chow SKH, Cui C, Liu C, Wang Q, Chai S, Wong RMY, Zhang N and Cheung WH. Wnt/beta-catenin signaling pathway as an important mediator in muscle and bone crosstalk: a systematic review. J Orthop Translat 2024; 47: 63-73.
[24] Huybrechts Y, Mortier G, Boudin E and Van Hul W. WNT signaling and bone: lessons from skeletal dysplasias and disorders. Front Endocrinol (Lausanne) 2020; 11: 165.
[25] Zhu B, Xue F, Zhang C and Li G. Ginkgolide B promotes osteoblast differentiation via activation of canonical Wnt signalling and alleviates osteoporosis through a bone anabolic way. J Cell Mol Med 2019; 23: 5782-5793.
[26] Huang X, Li S, Lu W and Xiong L. Metformin activates Wnt/beta-catenin for the treatment of diabetic osteoporosis. BMC Endocr Disord 2022; 22: 189.
[27] Wang X, Tian Y, Liang X, Yin C, Huai Y, Zhao Y, Huang Q, Chu X, Wang W and Qian A. Bergamottin promotes osteoblast differentiation and bone formation via activating the Wnt/beta-catenin signaling pathway. Food Funct 2022; 13: 2913-2924.
[28] Zhao XL, Chen JJ, Zhang GN, Wang YC, Si SY, Chen LF and Wang Z. Small molecule T63 suppresses osteoporosis by modulating osteoblast differentiation via BMP and WNT signaling pathways. Sci Rep 2017; 7: 10397.