

miR-551a promotes osteogenic differentiation of BMSCs by regulating SKI

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ABSTRACT

Background: MiR-551a has been implicated in various biological processes, but its role in fracture healing remains unclear. This study aimed to investigate the role of miR-551a in fracture healing.

Objective: This study is intended to examine the regulatory role of miR-551a in fracture healing through its interaction with SKI.

Methods: The osteogenic differentiation potential of human bone marrow-derived mesenchymal stem cells (hBMSCs) was assessed through quantification of alkaline phosphatase activity using a commercial alkaline phosphatase (ALP) assay kit and measurement of osteogenic marker gene expression levels, including Osteocalcin (OCN), Runt-related transcription factor 2 (RUNX2), and Collagen I via RT-qPCR and Western blot experiments. A dual-luciferase reporter assay was utilized to examine the regulatory interaction between miR-551a and SKI.

Results: Serum levels of miR-551a were significantly elevated in treated fracture patients. Functional analysis revealed that miR-551a overexpression enhanced ALP activity and elevated expression of osteogenic markers, whereas miR-551a inhibition reduced ALP activity and downregulated these markers; SKI knockdown reversed the inhibitory effects of miR-551a suppression. Mechanistically, miR-551a directly targets and negatively regulates SKI.

Conclusions: In vitro evidence suggests that miR-551a may promote osteogenic differentiation of hBMSCs by directly targeting and downregulating SKI, which may be relevant to fracture repair.

Keywords: Fracture healing, miR-551a, SKI, ALP, hBMSCs.

INTRODUCTION

Traumatic fracture refers to the disruption of bone integrity or continuity due to various external forces or mechanical factors, representing a common condition in orthopedics and emergency medicine with primary etiologies including trauma, osteoporosis, and infection¹. Following fracture occurrence, concomitant injuries to muscles, blood vessels, ligaments, skin, or nerves frequently manifest alongside significant impairment of limb function². Fracture healing constitutes a sophisticated regenerative process, with studies indicating delayed union or nonunion occurring in approximately 5% of cases^{3,4}. The primary objectives of fracture management are restoration of anatomical alignment, provision of stable fixation, and promotion of biological healing to maximize functional recovery⁵. Foundational principles include anatomical reduction and fracture stabilization, complemented by biological augmentation strategies

such as rhBMP-2 (FDA-approved for open tibial fractures), which accelerates callus formation by inducing osteogenic differentiation of stem cells⁶. In recent orthopedic research, BMSCs have emerged as promising seed cells⁷, demonstrating potent osteogenic potential under combined osteoinductive factors and biomechanical stimulation in clinical studies⁸. The therapeutic potential of bone marrow-derived approaches for fracture healing has also been demonstrated in clinical settings, particularly for managing delayed union and nonunion⁹.

Accumulating evidence indicates that microRNAs (miRNAs) play crucial regulatory roles in bone healing by modulating osteoblast function and maintaining osteoclast-osteoblast balance¹⁰. Specifically, miR-26a/CD34-exos exosomes protect against glucocorticoid-induced osteonecrosis of the femoral head through osteogenic induction¹¹, while miR-26a/b promotes osteogenic differentiation of unrestricted somatic stem cells by targeting osteogenic inhibitors¹².

Notably, miR-148a-3p demonstrates protective effects against osteonecrosis of the femoral head (ONFH)¹³. Although miR-551a has been studied in cancer contexts, its role in bone healing remains largely unexplored¹⁴. Notably, one study reported its downregulation in atrophic nonunion fractures, suggesting a potential involvement in fracture repair¹⁵. However, the mechanistic basis underlying miR-551a's involvement in fracture pathology remains unexplored, necessitating further investigation into its role in skeletal repair. SKI, the cellular homologue of the v-ski oncogene, participates in pathophysiological processes across diverse cell types in multiple tissues¹⁶ and functions through bone morphogenetic protein 2 (BMP-2) and transforming growth factor beta (TGF- β) signaling pathways¹⁷. Elevated SKI expression is documented in degenerated intervertebral disc tissues¹⁸ and reportedly upregulated in osteonecrotic conditions¹⁹. Recently, the miR-26a/SKI axis was reported to regulate osteogenic differentiation of mouse BMSCs²⁰. However, whether other miRNAs, particularly miR-551a, modulate SKI to influence osteogenic differentiation of human BMSCs (hBMSCs) in the context of fracture healing remains unknown.

Our in vitro findings demonstrate that miR-551a enhances the osteogenic potential of hBMSCs by modulating SKI expression, and thus may promote fracture repair, suggesting a potential role for this axis in fracture repair.

MATERIALS AND METHODS

Patient's data

This study enrolled 85 traumatic fracture patients who underwent surgical internal fixation at Xuzhou Renci Hospital between April 2023 and March 2025 as the experimental group. Fracture locations were documented from preoperative radiographs, including the tibia, femur, humerus, and forearm bones (radius/ulna). According to the AO/OTA classification, all fractures were categorized as type A (n=56) or type B (n=29). Patients received internal fixation using either intramedullary nails (n=41) or locking compression plates (n=44), with the choice of implant made by the attending surgeon based on fracture pattern and soft tissue condition. Clinical healing was defined as pain-free full weight-bearing and radiographic evidence of callus bridging across the fracture site on both anteroposterior and lateral views. According to this definition, all 85 patients achieved clinical healing within 3 to 6 months post-surgery, and no cases of

delayed union or nonunion were documented during the follow-up period (ranging from 6 to 12 months).

Blood samples were collected at admission (pre-treatment) and at postoperative days 1, 7, 14, and 21. Additionally, 85 age-matched healthy volunteers undergoing physical examinations during the same period were recruited as controls. Inclusion criteria for fracture patients were: 1) radiologically confirmed traumatic fracture with definitive injury mechanism; 2) closed simple fractures of long bones (e.g., limbs); 3) meeting surgical indications and receiving internal fixation at our institution. Exclusion criteria comprised: 1) concomitant traumatic brain injury or consciousness disorders; 2) open/pathological fractures; 3) fractures with visceral hemorrhage or multi-organ dysfunction; 4) coagulation abnormalities or malignancies.

Approval was obtained from the ethics committee of Xuzhou Renci Hospital. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. All participants provided written informed consent for blood sample collection used in this research.

Bioinformatics

The miRNA expression profiles from GSE93083 (GEO database: www.ncbi.nlm.nih.gov/geo/) were analyzed to compare fracture patients versus healthy controls. The specific details of GSE93083 include: the samples consist of peripheral blood from fracture patients, analyzed using platform GPL8786, with a total of 9 fracture patients and 4 healthy controls (age range 30–59 years, male/female). Data processing was performed using NCBI GEO2R. Differential expression analysis was conducted with thresholds of fold change > 1 and P-value < 0.05.

Target genes were predicted using starBase (binding sites: <https://rnasysu.com/encori/index.php>), TargetScan (conserved targets: www.targetscan.org/), and miRDB (functional prediction: <https://mirdb.org/>). GO/KEGG analyses were conducted via <http://www.bioinformatics.com.cn/>, with miRNA-gene interactions verified by starBase.

Blood sampling

Peripheral venous blood (5 mL) was collected from fracture patients at the following time points: at hospital admission (pre-treatment), and at 1 day, 7 days, 14 days, and 21 days post-surgery. Blood was also collected from healthy volunteers during routine physical examinations. The blood was drawn using BD Vacutainer® serum separator tubes (SST). After

30 minutes of clotting at room temperature, samples were centrifuged at $3500 \times g$ for 10 minutes, followed by serum storage at -80°C prior to analysis.

Cell activation and transfection

hBMSCs (Cyagen, USA) were cultured in Gibco's complete medium for adult human bone marrow mesenchymal stem cells (Gibco, USA), comprising Gibco basal medium supplemented with 10% FBS (Invitrogen, USA), 1% penicillin-streptomycin (Gibco, USA), and 2 mM L-glutamine (Gibco, USA). Cells were routinely passaged every 3 days using 0.25% trypsin-EDTA solution (Gibco, USA) and maintained at passages 3-5 during logarithmic growth for subsequent experimentation. All cell lines were authenticated using short tandem repeat (STR) profiling and tested negative for mycoplasma contamination.

hBMSCs were seeded at a density of 2×10^5 cells per well in 6-well plates and maintained for 24 hours in DMEM supplemented with 10% fetal bovine serum (FBS) under standard culture conditions of 37°C and 5% CO_2 in a humidified incubator. Upon reaching 75-85% confluency in confocal microscopy culture dishes, cells were co-transfected using Lipofectamine 2000 (Invitrogen, USA) with either si-NC (50nM), si-SKI (50nM), mimic NC, miR-551a mimic (100nM), inhibitor NC, or miR-551a inhibitor (100nM) in serum-free DMEM. The miR-551a mimic, miR-NC, and miR-551a inhibitor were provided by Shanghai GenePharma Co., Ltd (Table I). Following a 4h transfection incubation period, the medium was replaced with complete DMEM growth medium.

Osteogenic induction

hBMSC were seeded in 6-well plates at a density of 5×10^4 cells per well. Upon reaching 60%-70% confluency, the culture medium was replaced with osteogenic induction medium (HUXMA-90021, Cyagen, USA) consisting of basal medium supplemented with 10% FBS, 0.1 μM dexamethasone, 10 mM β -glycerophosphate, 50 μM ascorbic acid, and 1% penicillin-streptomycin. The induction medium

was refreshed every 3 days for 14 days prior to subsequent analyses.

Reverse transcription quantitative real-time PCR (RT-qPCR)

Total RNA was isolated from serum and cell samples using Trizol reagent (TransGen, China) according to standardized protocols. miRNA analysis employed stem-loop RT-qPCR methodology. Briefly, 1000 ng of total RNA was reverse-transcribed with stem-loop RT primers using the miRNA First-Strand cDNA Synthesis Kit (Vazyme, China). Subsequent qPCR amplification utilized miRNA Universal SYBR® qPCR Master Mix (Vazyme, China) per manufacturer's specifications, with small nuclear RNA U6 serving as the endogenous control. Custom primers included: miR-551a forward primer: (5'-GCGACCCACTCTTGGTTTCCA-3') and reverse primer: (5'-CAGTGCGTGTCTGAGT-3'). U6 forward, 5'-CTCGCTTCGGCAGCACATA-3', and U6 reverse 5'-CGCTTCACGAATTTGCGTG-3'. For mRNA quantification, first-strand cDNA synthesis was performed using TransScript® II SuperMix (TransGen Biotech, China), followed by qPCR performed with TransStart® Top Green SuperMix (TransGen Biotech, China) on a thermal cycler. Gene expression levels were measured using GAPDH as the endogenous reference gene. Primer sequences were as follows: SKI forward, 5'-GAGAAATTCGACTATGGCAACAAG-3', and reverse 5'-GGCCGAAATCAGATACTTTAAC-3'. GAPDH forward, 5'-GGAGCGATCCCTCCAAAAT-3', and reverse 5'-GGCTCTTGTCTACTTCTCATGG-3'. All reactions were executed in technical triplicate, with relative quantification determined via the $2^{-\Delta\Delta\text{Ct}}$ method.

Western Blot

Total proteins were extracted from hBMSCs using RIPA buffer (#P0013B, Beyotime Biotechnology). Protein concentrations were determined using a BCA protein assay kit (#23225, Thermo Fisher Scientific, USA). Equal amounts of protein were

Table I. — Sequences used in this study.

Name	sequence (5'-3')
hsa-miR-551a mimic	5'-GCGACCCACUCUUGGUUCCA-3'
hsa-miR-551a inhibitor	5'-GAAACCAAGAGUGGGUCGCUU-3'
mimic NC	5'-UUCUCCGAACGUGUCACGUTT-3'
inhibitor NC	5'-ACGUGACACGUUCGGAGAATT-3'
si-SKI	5'-CAGAAGAATGGTACAAATCCAAG-3'

mixed with SDS-PAGE loading buffer, heated at 100°C for 5 min, separated by 10% SDS-PAGE, and blotted onto PVDF membranes (Millipore, MA, USA). Membranes were blocked with 5% nonfat milk for 1 h at room temperature, washed with TBST, and incubated overnight at 4°C with primary antibodies against Runx2 (#ab236639, Abcam, USA), osteocalcin (#ab133612, Abcam, USA), type I collagen (#ab260043, Abcam, USA), and GAPDH (#5174, Cell Signaling Technology, USA). After TBST washing, membranes were incubated with HRP-conjugated goat anti-rabbit secondary antibodies (#7074, Cell Signaling Technology, USA) for 1 h at room temperature. Protein bands were visualized using an electrochemiluminescence detection kit (Amersham).

Detection of alkaline phosphatase (ALP) activity

Following osteogenic induction, the culture medium was carefully aspirated, and cells were lysed using 50mM Tris-HCl, 150mM NaCl, and 1% Nonidet P-40 detergent solution (Nacalai Tesque, Japan). Lysates were centrifuged at $12,000 \times g$ for 3 minutes at 4°C to obtain clarified supernatants. ALP activity was spectrophotometrically quantified at 405 nm wavelength after 30-minute incubation with a commercial ALP assay kit (Takara Bio, Japan), and ALP activity was normalized to total protein measured with the BCA protein assay method. Expressed as optical density units per milligram of cellular protein.

Molecular markers of osteogenic differentiation

To validate the osteogenic function of the miR-551a-SKI axis, RT-qPCR was performed to analyze osteogenic differentiation markers Osteocalcin (OCN), Runt-related transcription factor 2 (RUNX2), and Collagen I, normalized to GAPDH. The primer sequences were as follows: OCN forward, 5'-CCTGAGTCTGACAAAGCCTTC-3', and reverse 5'-GCGGTCTTCAAGCCATACTG-3'. RUNX2 forward, 5'-CGTCCACTGTCACTTTAATAGCTC-3', and reverse 5'-GTAGCCAGGTTCAACGATCTG-3'. Collagen I forward, 5'-CTGGCGGTTTCAGGTCCAAT-3', and reverse 5'-TCCAAACCACTGAAGCCTCG-3'.

Luciferase activity assay

Using starBase to predict the miR-551a-SKI interaction, fragments of the SKI 3'-UTR containing

either wild-type (WT) or mutated (MUT) miR-551a binding sequences were cloned into the pmirGLO Dual-Luciferase Vector (Promega, USA), generating SKI-WT and SKI-MUT constructs, followed by co-transfection of HEK293T cells (seeded at 5×10^6 cells/dish) with miR-551a inhibitor or miR-551a mimic alongside SKI-WT/SKI-MUT plasmids using Lipofectamine 3000, with luciferase activity subsequently quantified via the Dual-Luciferase Reporter Assay System.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 10.1.2. Data from at least three independent experiments were expressed as mean $\pm$ standard deviation (SD). Comparisons between two groups were analyzed using unpaired two-tailed Student's t-test. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons was applied. Two-way ANOVA followed by Šidák's post hoc test was used for analyses involving two independent variables. A $P < 0.05$ was considered statistically significant.

RESULTS

Differential serum expression of miR-551a in traumatic fracture

Bioinformatic analysis revealed significantly upregulated serum miR-551a levels in fracture patients versus healthy controls ($P < 0.001$, $\log_2 FC > 2.5$), as demonstrated in the volcano plot (Supplementary Figure 1), thus leading to its selection for further investigation; subsequent PCR assays confirmed that miR-551a expression was markedly higher in fracture patients after treatment than in healthy controls (Figure 1A) ($P < 0.001$).

To investigate the relationship between miR-551a and fracture healing, we monitored its expression levels at the time of injury and at 1 day, 7 days, 14 days, and 21 days post-treatment. The results showed that serum miR-551a levels increased progressively from day 1 to day 14 post-surgery, peaked at day 14, and then declined gradually through day 21 (Figure 1B).

miR-551a regulates osteogenic differentiation in hBMSCs

To investigate the relationship between miR-551a and osteogenic differentiation, we monitored its expression during hBMSC differentiation, revealing

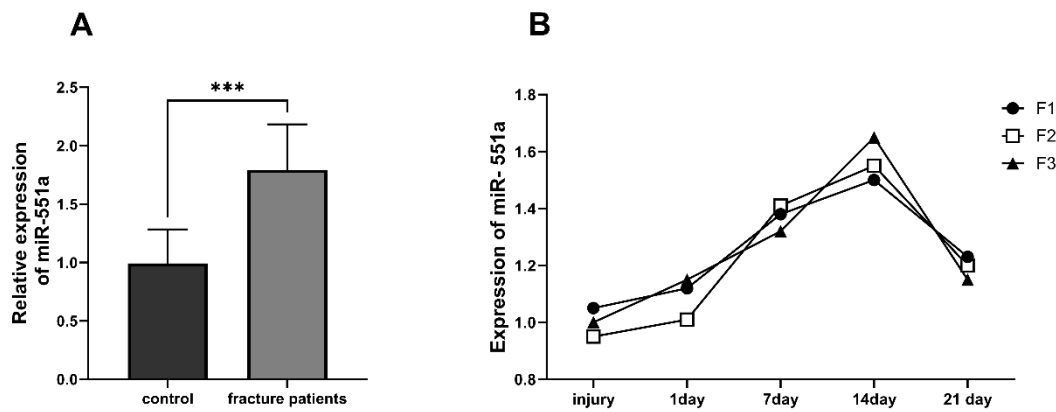


Fig. 1 — MiR-551a expression levels. (A) MiR-551a expression levels between healthy controls and fracture cohorts in serum. (B) MiR-551a expression levels after injury and on postoperative days 1, 7, 14, and 21. *** $P < 0.001$.

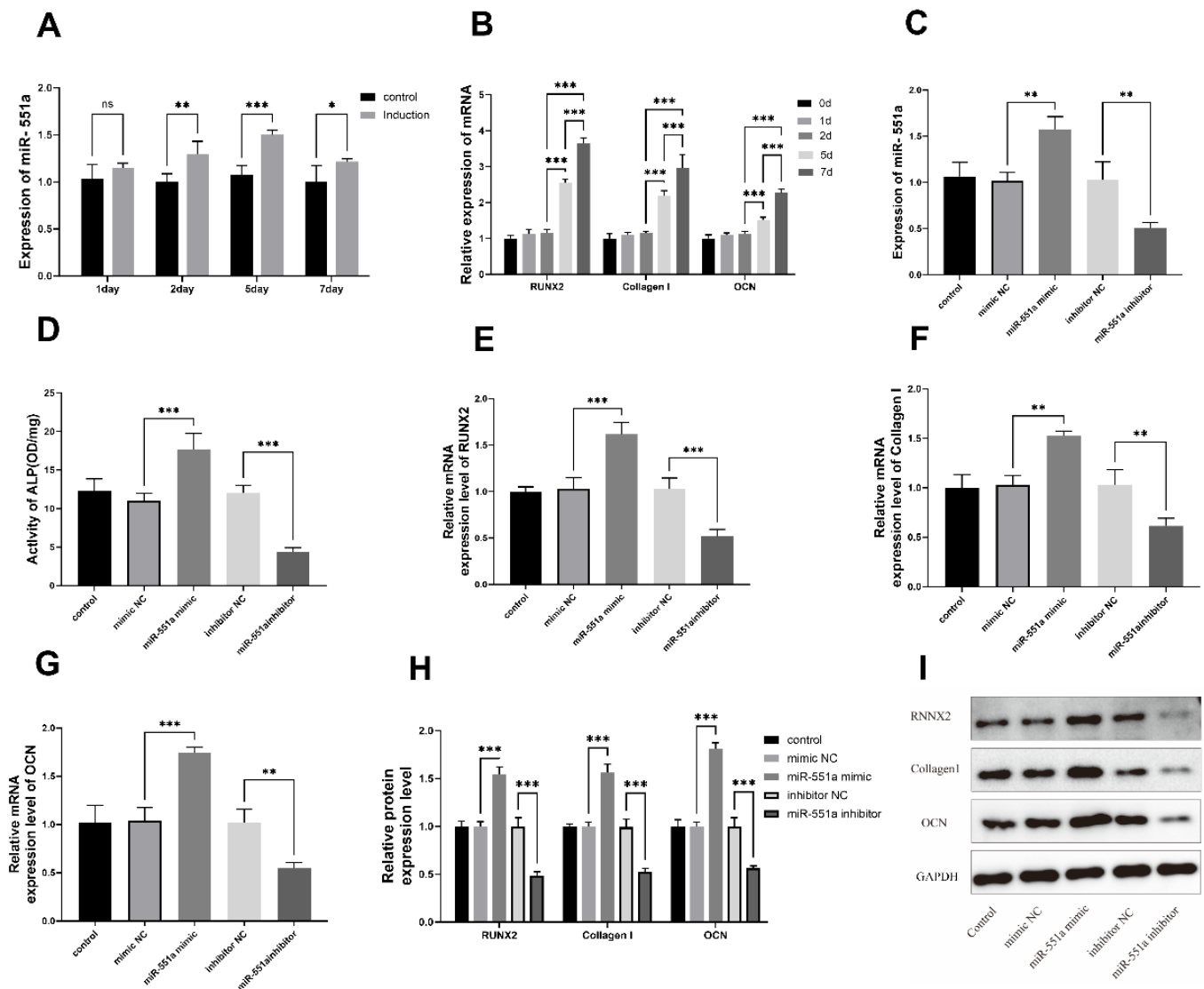


Fig. 2 — MiR-551a modulates osteogenic differentiation. (A) Association between miR-551a expression levels and osteogenic differentiation. (B) RUNX2, Collagen I, and OCN expression levels on postoperative days 0, 1, 2, 5, and 7. (C) Transfection of miR-551a into hBMSCs. (D) ALP expression levels. (E) RUNX2 expression levels. (F) Collagen I expression levels. (G) OCN expression levels. (H-I) RUNX2, Collagen I, and OCN protein expression levels. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

significantly elevated levels versus undifferentiated controls ($P < 0.05$) (Figure 2A). Subsequently, we examined the expression of osteogenic differentiation markers during hBMSC differentiation. The results demonstrated that, with prolonged culture time, the mRNA expression levels of RUNX2, Collagen I, and OCN exhibited significant upregulation starting from the fifth day, suggesting that miR-551a expression may facilitate progression from early to late stages of osteogenic differentiation and the acquisition of a mature osteoblast phenotype, thereby influencing fracture healing ($P < 0.001$, Figure 2B).

To investigate the effect of miR-551a on fracture healing, hBMSCs were transfected with negative control mimic (mimic NC), miR-551a mimic, negative control inhibitor (inhibitor NC), or miR-551a inhibitor, demonstrating that the miR-551a mimic significantly upregulated, whereas the inhibitor downregulated, miR-551a expression (all $P < 0.01$) (Figure 2C); moreover, the miR-551a mimic enhanced ALP activity ($P < 0.001$) and the mRNA and protein expression levels of RUNX2 ($P < 0.001$), Collagen I ($P < 0.01$), and OCN ($P < 0.001$), whereas the inhibitor exerted opposite effects (all $P < 0.01$) (Figures 2D-I). These in vitro results suggest that miR-551a may promote osteogenic differentiation of hBMSCs, which is a key cellular process in fracture healing.

miR-551a directly targets SKI

To elucidate the mechanism of miR-551a in hBMSC osteogenesis, GO enrichment analysis showed that the predicted target genes of miR-551a were significantly enriched in terms related to osteogenic differentiation, including “regulation of osteoblast differentiation” (GO:0045667, $P < 0.001$, Figure 3A), where SKI, YAP1, CDK6, IGFBP5, MEF2C, PTK2, and IL6ST emerged as associated targets. As SKI has been reported in the literature to regulate osteogenic differentiation, an investigation of Gene Ontology (GO) terms associated with SKI in the UniProt database revealed that SKI is involved in the negative regulation of osteoblast differentiation (GO:0045668)²⁰. We detected the expression levels of SKI in the serum of both healthy controls and fracture patients. The results revealed that, compared with healthy controls, the mRNA expression level of SKI in fracture patients was significantly decreased ($P < 0.001$) (Figure 3B).

Therefore, SKI was prioritized for subsequent mechanistic studies. We transduced hBMSCs with mimic NC, miR-551a mimic, inhibitor NC, and miR-551a inhibitor. Results showed that compared to mimic

NC, SKI mRNA and protein expression levels were significantly decreased in the miR-551a mimic group (all $P < 0.05$), while in the miR-551a inhibitor group, SKI expression levels were significantly elevated compared to inhibitor NC (all $P < 0.001$) (Figure 3C-E). Furthermore, miR-551a inhibitor significantly increased luciferase activity in hBMSCs transfected with the SKI-WT construct ($P < 0.01$), whereas the miR-551a mimic significantly decreased luciferase activity in these cells ($P < 0.001$). However, neither the inhibitor nor the mimic had a significant effect on hBMSCs transfected with the SKI-MUT construct (Figure 3F). These results indicate that miR-551a directly targets and negatively regulates SKI.

miR-551a regulation of SKI expression impacts osteogenic differentiation

To investigate whether the regulation of SKI by miR-551a affects osteogenic differentiation, si-NC and si-SKI were transfected into hBMSCs, respectively. The results showed that the expression of SKI in the si-SKI group was significantly lower than that in the si-NC group ($P < 0.01$) (Figure 4A).

Subsequent transfection of hBMSCs with inhibitor NC, miR-551a inhibitor, miR-551a inhibitor + si-NC, or miR-551a inhibitor + si-SKI. The results demonstrated that compared to inhibitor NC, miR-551a inhibitor transfection markedly decreased levels of ALP activity ($P < 0.001$), and the mRNA and protein expression levels of RUNX2 ($P < 0.001$), OCN ($P < 0.01$), and Collagen I ($P < 0.01$), whereas compare to miR-551a inhibitor + si-NC, co-transfection with miR-551a inhibitor + si-SKI reversed these effects (all $P < 0.05$) (Figures 4B-G). These findings indicate that inhibition of miR-551a significantly downregulates the generation of osteoblast biomarkers, and this process is restored following SKI inhibition, suggesting that miR-551a affects osteogenic differentiation of hBMSCs by targeting SKI.

DISCUSSION

Traumatic fractures primarily result from direct or indirect trauma, causing disruption of bone integrity or continuity, with considerable variability in prognosis. Delayed fracture healing is defined as the failure to achieve healing criteria within the conventional timeframe²¹, imposing substantial economic burdens on patients' families while increasing risks of secondary surgeries and poor outcomes.

BMSCs, possessing robust proliferative capacity and multipotent differentiation potential²², facilitate

fracture healing by promoting angiogenesis through the secretion of growth factors, including BMPs and VEGF, and differentiating into osteoblasts to generate new bone tissue²³. Osteoblast differentiation is a pivotal determinant of fracture healing efficacy, directly dictating the rate of callus formation, mineralization quality, and ultimate biomechanical competence²⁴, thereby underscoring the critical necessity for dedicated research in this domain. In this context, the present study investigated the role of miR-551a in osteogenic differentiation of hBMSCs. The findings demonstrate for the first time that miR-551a directly targets SKI to promote osteogenic differentiation of hBMSCs in the context of fracture healing, based on *in vitro* experimental evidence.

Our study found that serum miR-551a levels were significantly higher in fracture patients after surgical treatment than in healthy controls. To investigate the expression changes of miR-551a during fracture healing, we observed that, compared with healthy controls, the expression level of miR-551a began to increase after fracture and further elevated after treatment. Continuous detection over 21 days showed that miR-551a reached its peak level on day 14, followed by a gradual decline, returning to near-normal levels by day 21. These results suggest that serum miR-551a levels correlate with the time course of fracture healing and may play a significant role in this process. To further explore the impact of miR-551a on fracture healing, we conducted *in vitro* cell experiments to examine the effect of miR-551a expression on osteogenic markers. The results showed that increased miR-551a expression enhanced the expression of ALP, RUNX2, Collagen I, and OCN in hBMSCs, indicating that miR-551a promotes osteogenic cell differentiation. Studies have shown that nonunion is associated with impaired osteogenic differentiation. Wei et al. found that nonunion may result from reduced miR-551a expression, leading to blocked osteogenic differentiation²⁵, which is consistent with the observation that miR-551a promotes osteogenic differentiation while its expression is decreased in nonunion patients. Similarly, a recent study reported that downregulation of miR-656-3p promotes osteogenic differentiation by targeting BMP-2²⁶. Collectively, these findings suggest that early upregulation of miR-551a reflects active osteogenic differentiation, whereas its downregulation in chronic nonunion may contribute to impaired bone repair.

MicroRNAs mediate mRNA expression through translational regulation^{27,28}. To further investigate the

potential mechanisms by which miR-551a influences osteogenic differentiation, we identified downstream target genes regulated by miR-551a. Studies have demonstrated that SKI can modulate BMP-2 and TGF- β signaling pathways²⁹, with evidence indicating that the TGF- β /Runx2 axis mediates impairment in mechanically-induced osteogenic differentiation³⁰. Additional findings demonstrate that TGF- β receptor type I regulates the expression of osteogenic markers³¹. SKI has been established as a potent negative regulator of TGF- β /Smad signaling in various cellular contexts. Mechanistically, SKI directly interacts with Smad2, Smad3, and Smad4, recruiting transcriptional repressor complexes such as N-CoR and HDACs to TGF- β -responsive promoters³². Furthermore, SKI can suppress Smad2 phosphorylation by the activated TGF- β type I receptor, representing an additional layer of inhibition³³. In chondrocytes, SKI overexpression inhibits TGF- β /Smad3 signaling and accelerates hypertrophic differentiation³⁴.

It should be noted that the source of circulating SKI mRNA remains to be determined. SKI is widely expressed in various tissues, including bone and bone marrow³⁵, and may be released from multiple cellular sources during fracture healing. Additionally, while our study focused on SKI as a direct target of miR-551a, other known miR-551a targets such as PRL-3³⁶ and MEF2C³⁷ have been identified in different contexts. Although these targets have not been validated in hBMSCs or fracture healing, we cannot exclude that miR-551a may exert effects through additional downstream targets.

Therefore, we detected SKI expression levels in the serum of fracture patients and found that SKI expression was decreased compared to healthy controls. Notably, SKI inhibition partially restored the expression of osteogenic markers impaired by miR-551a knockdown. This suggests that miR-551a may regulate osteogenic cells through SKI. To confirm the regulation of SKI by miR-551a, we transfected miR-551a mimic and miR-551a inhibitor to detect SKI mRNA and protein levels. Results showed that, compared with the control group, miR-551a mimic decreased SKI expression, while miR-551a inhibitor increased SKI expression. Dual-luciferase reporter gene assay confirmed that miR-551a directly targets and inhibits SKI.

This study also has some limitations. First, all data presented here are derived from *in vitro* experiments, as this study focused on mechanistic validation at the cellular level. Therefore, we have not yet established that miR-551a promotes fracture healing *in vivo*. The

conclusions are currently applicable only to the *in vitro* setting. Future work using rodent fracture models is needed to confirm the role of the miR-551a/SKI axis in bone repair. In addition, during the 14-day osteogenic induction period, the number of transfections may also influence the expression of miR-551a, which requires further investigation. Furthermore, evaluation of mineralized nodule formation (e.g., Alizarin Red S staining) was omitted from this exploratory study due to practical limitations, which restricts our ability to assess late-stage osteogenic differentiation. Finally, the specific downstream mechanisms by which SKI regulates osteogenic differentiation may involve other signaling pathways, and the underlying mechanisms require further investigation.

CONCLUSIONS

Taken together, these *in vitro* findings suggest that miR-551a may promote osteogenic differentiation of hBMSCs through SKI suppression, which may in turn facilitate fracture healing.

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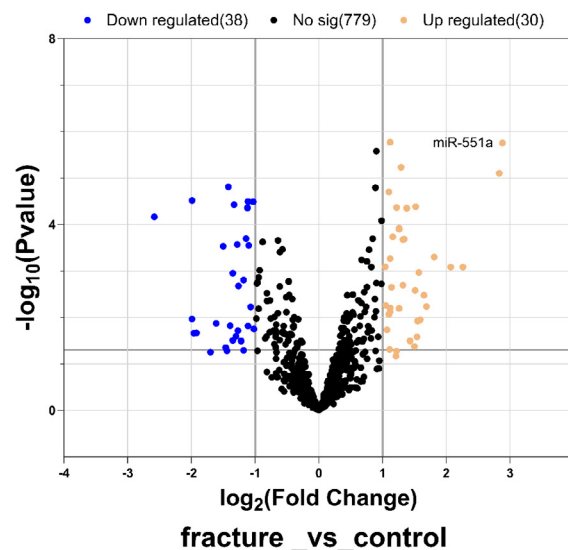
Conflict of Interest: The authors declare no conflicts of interest.

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SUPPLEMENT



Supplementary fig. 1 — Volcano plot of serum miRNA profiles from fracture subjects.