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Dysregulation of miR-106a-5p/PTEN axis associated with progression and diagnostic of postmenopausal osteoporosis

Xiangjie Liu[1†], Xiaogang Zhang[2†] and Meini Cen[3,4*]

Abstract

Objective Postmenopausal osteoporosis (PMOP) is a bone disorder in postmenopausal women and a significant risk factor for fragility fractures. This study aims to explore the role of miR-106a-5p in the pathogenesis of PMOP and its potential as a diagnostic biomarker.

Methods 220 postmenopausal women were recruited. The levels of miR-106a-5p, PTEN, and osteogenic-related genes were quantified using qRT-PCR. The relative protein of PTEN was detected using Western blotting. ROC curve and Pearson correlation were employed to evaluate the diagnostic value and relationships between variables. To model iron accumulation, hFOB1.19 osteoblasts were treated with ferric ammonium citrate (FAC). Cell proliferation and apoptosis were assessed using the CCK-8 and flow cytometry. The target relationship was verified using dual-luciferase assays.

Results miR-106a-5p levels were reduced, while PTEN levels were increased in PMOP. miR-106a-5p was positively correlated with bone mineral density and negatively correlated with ferritin. In the FAC-treated cells, miR-106a-5p decreased, and PTEN increased. Dual-luciferase assays confirmed that miR-106a-5p targets PTEN. Successful transfection was confirmed by observing the corresponding changes in miR-106a-5p and PTEN expression. Up-regulated miR-106a-5p increased the PTEN protein level, mRNA expression of RUNX2, OPN, and OCN, promoted cell proliferation, and decreased cell apoptosis under iron accumulation conditions. These effects were reversed by the upregulation of PTEN.

Conclusion miR-106a-5p has the potential to diagnose osteoporosis in postmenopausal women and is linked to ferritin levels. miR-106a-5p plays a protective role in PMOP by regulating PTEN under conditions of iron accumulation, suggesting its potential as a promising biomarker for PMOP.

Keywords miR-106a-5p, PTEN, Postmenopausal osteoporosis, Iron accumulation

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Introduction

Postmenopausal osteoporosis (PMOP) is a prevalent bone metabolism disorder affecting postmenopausal women [1, 2]. It results from an imbalance between osteoclast-driven bone resorption and osteoblast-mediated bone formation, disrupting bone homeostasis [3, 4]. This leads to reduced bone mass, deteriorated microarchitecture, increased fragility, and a higher risk of fractures [5]. With the aging population, the incidence of this age-related condition is rising [6]. According to a 2018 epidemiological survey, osteoporosis poses a major health concern for middle-aged and elderly Chinese women, particularly postmenopausal women, with significantly higher incidence rates than men [6, 7]. Among women over 65, the prevalence exceeds 50%. Key risk factors include aging, ongoing calcium loss, and hormone imbalance levels [2, 8]. Recent research has increasingly linked iron accumulation to disrupted bone metabolism and bone mass reduction in postmenopausal women [9]. Normally, iron is lost through feces, skin, and menstruation, but postmenopausal women no longer experience menstrual iron loss [10]. Consequently, serum ferritin—an indicator of iron status—can increase two- to threefold, reflecting a substantial rise in total body iron stores during this period [10, 11]. Iron accumulation impacts both bone formation and resorption, suggesting that managing iron overload could be a critical strategy for preventing and treating PMOP.

MicroRNAs (miRNAs) are short (approximately 20 nucleotides) single-stranded non-coding RNAs [12, 13]. In osteoporosis (OP), numerous miRNAs have been found to be dysregulated in both bone tissue and circulation, contributing to disease development [14–16]. miR-34a-5p, miR-9-5p, and miR-98-5p were screened from sequencing data and validated in PMOP samples, demonstrating strong diagnostic value through receiver operating characteristic (ROC) analysis [17]. Serum levels of miR-148a were found to be positively correlated with β-CTX (a serum osteoporotic marker) in PMOP patients and in vitro experiments further revealed that upregulation of miR-148a enhances osteoclast-mediated bone resorption, suggesting its potential as a therapeutic target for PMOP [18–20]. Additionally, miR-187-3p was shown to be downregulated in the femur of an osteoporosis mouse model, and under mechanical stress, miR-187-3p was significantly involved in osteoblast differentiation and contributed to the improvement of OP [21]. These findings underscore the critical roles of miRNAs in OP pathogenesis and their potential in regulating bone homeostasis. Recent research has also shown that miR-106a-5p, derived from osteoclasts, can stimulate osteoblast differentiation through intercellular signaling [22]. Some studies have incorporated miR-106a-5p into competing endogenous RNA networks that influence osteoclast differentiation [23]. A sequencing study suggests that this miR-106a-5p may influence osteoblast differentiation by regulating the proliferation of mesenchymal stem cells [24]. However, its expression in OP patients and functional role in human-derived osteoblasts remain unclear. miRNAs typically exert their effects by binding to the 3'UTR region of mRNAs to regulate gene translation and expression [25]. Bioinformatic analysis suggests that miR-106a-5p may target PTEN, a gene located at 10q23.31 and known to be involved in osteoblast differentiation [26]. For instance, PTEN expression is downregulated during osteogenic differentiation in aged rats [27]. Data mining suggested that PTEN may be a key gene in the ferroptosis signaling pathway using high-throughput sequencing data [28]. Given that serum ferritin levels—an indicator of iron load—are elevated in osteoporotic individuals, it is hypothesized that miR-106a-5p may influence OP pathogenesis through its regulation of PTEN and be associated with iron accumulation in OP.

In summary, the study aims to investigate the function of the miR-106a-5p/PTEN axis in PMOP. By establishing an iron overload cell model, the relationship between this regulatory axis and iron accumulation in PMOP was explored. The findings provide valuable insights into the mechanisms underlying the onset and progression of PMOP.

Materials and methods

In the clinical study, serum samples from PMOP patients and healthy controls were analyzed to measure miR-106a-5p expression and its correlation with bone mineral density (BMD) and ferritin levels. This provides initial clinical evidence for the diagnostic relevance of miR-106a-5p and its potential association with iron accumulation. In the cellular study, in vitro experiments using the human osteoblast cell line under iron overload conditions were conducted to investigate the functional role of miR-106a-5p. These included assessments of cell proliferation, apoptosis, osteogenic gene expression, and PTEN protein levels. The dual-luciferase assays were conducted to validate the interaction between miR-106a-5p and PTEN.

Volunteer recruitment

From March 2020 to March 2022, 220 postmenopausal women (including 108 PMOP patients in the PMOP group and 112 healthy volunteers in the control group) were recruited from the Physical Examination Center and the Department of Orthopedics at The First Affiliated Hospital of Chengdu Medical College. Fasting venous blood was collected either on the day of the physical examination or the second day of admission. The blood was analyzed using an automated hematology analyzer. Ferritin levels were measured using an ELISA method.

All volunteers were able to independently provide consent and signed informed consent forms. Approval was obtained from the ethics committee of The First Affiliated Hospital of Chengdu Medical College (2020CYFY-HEC021). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Inclusion Criteria: (1) Age between 50 and 70 years; (2) No history of infection, trauma, surgery, or use of anti-inflammatory drugs in the past two weeks; (3) No history of long-term treatment with glucocorticoids, estrogen, calcitonin, or bisphosphonates; (4) No acute cardiovascular or cerebrovascular diseases, blood donation, transfusion, or iron treatment within the past year.

OP Diagnostic Criteria: BMD at the femoral neck was measured using dual-energy X-ray absorptiometry. A T-score greater than −1 was considered normal bone mass, while a T-score of less than −2.5 was diagnostic of OP.

Cell culture and transfection

The human-derived osteoblast hFOB1.19 was obtained from the American Type Culture Collection (ATCC, USA; Catalog No. CRL-3602) and cultured in DMEM/F12 medium (Thermo, USA) supplemented with 10% fetal bovine serum (Thermo). Only cells within 10 passages were used for experiments. To induce iron accumulation, 100 μmol/L ferric ammonium citrate (FAC) was added based on previous experimental protocols [29].

1×10^5 cells were seeded per well in 24-well plates. When the cells reached 70% confluence, transfection was performed using Lipofectamine 2000 (Invitrogen). miR-mimics and negative controls (NC) were purchased from Genepharma (Shanghai, China). Overexpression plasmids carrying PTEN sequences were constructed using the pcDNA3.1 vector (Genepharma).

The cells were divided into the following experimental groups: FAC group, FAC + miR-NC group, FAC + miR-mimic group, FAC + miR-mimic + oe-NC group, FAC + miR-mimic + oe-PTEN group.

CCK-8 assay

CCK-8 kit was purchased from Beyotime (Shanghai, China). 3000 transfected cells were seeded per well in a 96-well plate. At time points of 0, 24, 48, and 72 h, CCK-8 reagent was added to each well at a ratio of 10:1 (medium: CCK-8) and incubated for 2 h. Absorbance at 450 nm was measured using a Microplate Reader (Bio-Rad, USA).

Cell apoptosis assay

A suspension of 1×10^5 transfected cells was prepared using the Annexin V-FITC/PI Apoptosis Detection Kit (KeyGen, Jiangsu, China). The cells were incubated with the fluorescent dyes for 15 min in the dark. Apoptotic cells were then quantified using flow cytometry (FACS-Calibur, BD, USA).

Dual-luciferase reporter assay

Luciferase reporter constructs were provided by GenePharma. The PTEN sequence containing either wild-type or mutated miR-106a-5p binding sites was cloned into the pmirGLO vector (designated as WT-PTEN and MT-PTEN groups, respectively). hFOB1.19 cells were seeded in 24-well plates at a density of 5×10^4 cells per well and co-transfected with WT-PTEN or MT-PTEN plasmid along with 50 nM miR-106a-5p mimic or NC using Lipofectamine 2000. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, USA).

qRT-PCR

Total RNA, including miRNAs, was extracted using the TRIzol reagent (Thermo), with chloroform extraction and isopropanol precipitation steps to enhance RNA recovery. RNA purity was assessed based on OD 260/280 (1.9–2.1) and 260/230 (2.0-2.2). Reverse transcription was performed with 1000 ng of RNA using the PrimeScript RT Reagent Kit (Takara, Dalian, China). For miRNA-specific analysis, reverse transcribed was performed using the Mir-X™ miRNA First-Strand Synthesis Kit (Takara). PCR amplification was carried out with the TB Green Premix Ex Taq II kit (Takara) under the following conditions: 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH or U6 as the internal reference.

Western blot

Western blotting was performed to detect PTEN protein levels. Cells were lysed in lysis buffer supplemented with protease and phosphatase inhibitors (Beyotime) at 4 °C, followed by centrifugation at 12,000 rpm to collect the supernatant. Protein concentrations were determined using the Enhanced BCA Protein Assay Kit (Beyotime). Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membranes. After blocking, the membranes were incubated overnight at 4 °C with a primary antibody against PTEN (1:1000, Proteintech). Following three washes with PBS, membranes were incubated with an HRP-conjugated secondary antibody (1:4000, Proteintech) in blocking buffer (Beyotime) for 2 h at room temperature. GAPDH (1:5000, Proteintech) was used as a loading control. Protein bands were detected using an enhanced chemiluminescence (ECL) kit (Beyotime) and visualized with the Amersham Imager 600 system.

Table 1 Clinical and laboratory characteristics of the patients

Parameters	control ($n=135$)	PMOP ($n=142$)	*P* value
Age (year)	60.53 ± 5.53	60.00 ± 6.02	0.500
BMI (kg/m^2)	23.25 ± 2.03	23.62 ± 1.84	0.159
ALT (U/L)	15.04 ± 5.38	16.17 ± 6.58	0.167
AST (U/L)	18.18 ± 6.88	18.47 ± 7.85	0.769
ALP (U/L)	61.34 ± 11.40	62.02 ± 11.73	0.662
Cr (μmol/L)	60.54 ± 5.07	59.57 ± 6.21	0.208
BUN (mmol/L)	5.14 ± 0.96	5.15 ± 1.02	0.946
TC (mmol/L)	3.92 ± 0.66	3.97 ± 0.68	0.600
TG (mmol/L)	1.32 ± 0.29	1.38 ± 0.29	0.186
GLU (mmol/L)	5.27 ± 0.77	5.36 ± 0.73	0.405
Ca (mmol/L)	2.36 ± 0.13	2.37 ± 0.16	0.784
P (mmol/L)	1.11 ± 0.16	1.13 ± 0.19	0.358
BMD (g/cm^2)	0.86 ± 0.04	0.57 ± 0.03	< 0.001
Ferritin (ng/mL)	100.53 ± 22.89	171.99 ± 26.89	< 0.001

Abbreviations: ALT, Alanine transaminase; ALP, Alkaline phosphatase; AST, Aspartate Transferase; BMD, Bone mineral density; BMI, Body mass index; BUN, Blood urea nitrogen; Ca, Calcium; Cr, Creatinine; GLU, glucose; P, Phosphorus; TC, Total cholesterol; TG, Triglyceride; PMOP, Postmenopausal osteoporosis

Statistical analysis

Data are expressed as mean ± SD. The software R (version 4.4.0) and GraphPad Prism 9 were used to analyze data. The significance of the difference between the two groups was determined by unpaired Student' t test. Estimating the required sample size using power calculations (power.t.test function in R). The diagnostic value and relationship between variables were analyzed using ROC curves. And Pearson correlation. $P<0.05$ was considered statistically significant. All experiments were performed in triplicate.

Results

Clinical information

The clinical information of all volunteers is presented in Table 1. No significant differences were observed in age, Body mass index (BMI), Alanine transaminase (ALT), Aspartate Transferase (AST), ALP (Alkaline phosphatase), Cr (Creatinine), Blood urea nitrogen (BUN), Total cholesterol (TC), Triglyceride (TG), glucose (GLU), Calcium (Ca), or Phosphorus (P) levels (Table 1, $P>0.05$). However, BMD was significantly decreased and ferritin levels were elevated in the PMOP patients (Table 1, $P<0.05$).

miR-106a-5p decreased in the PMOP

The mean abundance of miR-106a-5p in PMOP patients was approximately 25% lower than in the control group (Fig. 1A, $P<0.05$). Receiver operating characteristic (ROC) curve analysis demonstrated that miR-106a-5p has good diagnostic performance, with an area under the curve (AUC) of 0.772 (Fig. 1B). Correlations between miR-106a-5p levels and clinical parameters are summarized in Table 2. miR-106a-5p was significantly positively correlated with BMD and negatively correlated with ferritin levels (Table 2, $P<0.05$). Based on the observed correlation between miR-106a-5p and ferritin, the iron accumulation cell model was established using FAC. Under FAC treatment, miR-106a-5p expression was markedly downregulated (Fig. 1C, $P<0.05$). These findings suggest that miR-106a-5p may serve as a potential biomarker for PMOP.

miR-106a-5p targeted PTEN

The binding site of miR-106a-5p on PTEN was predicted using the ENCORI database (Fig. 2A). This interaction was experimentally confirmed through dual-luciferase

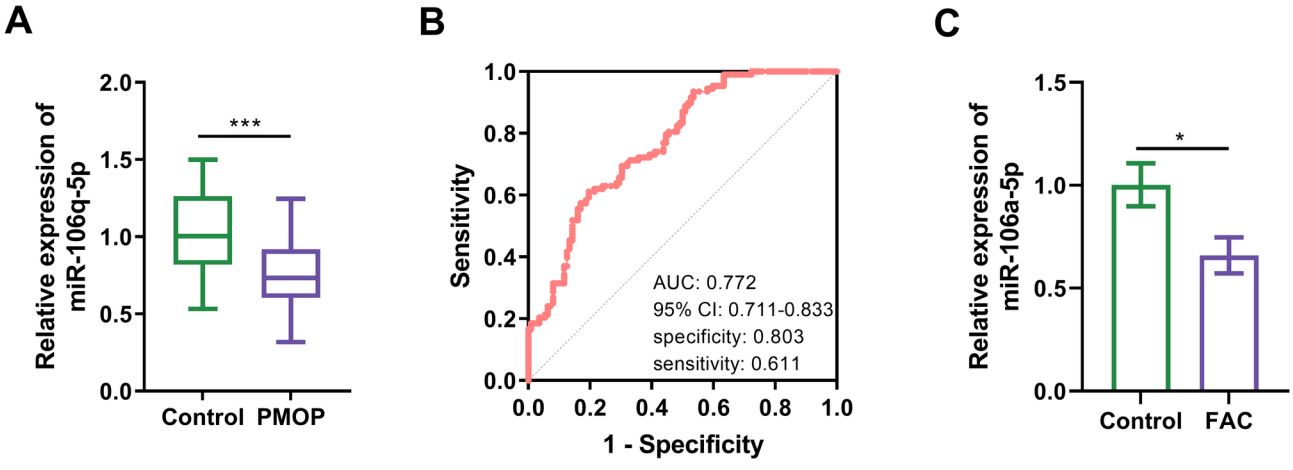

Fig. 1 Expression levels and diagnostic value of miR-106a-5p. (**A**) Expression levels of miR-106a-5p in control ($n=112$) and PMOP ($n=108$) groups. (**B**) Diagnostic value of miR-106a-5p as assessed by ROC curve. (**C**) Abundance of miR-106a-5p in FAC-treated hFOB1.19 cells ($n=3$). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. Control. FAC, ferric ammonium citrate; PMOP, postmenopausal osteoporosis; ROC, receiver operating characteristic

Table 2 Pearson correlation between miR-106a-5p clinical characters in PMOP

Parameters	coefficient	*P* value
Age (year)	0.011	0.906
BMI (kg/m^2)	-0.160	0.097
ALT (U/L)	-0.069	0.471
AST (U/L)	-0.176	0.068
ALP (U/L)	-0.086	0.374
Cr (μmol/L)	-0.062	0.518
BUN (mmol/L)	-0.024	0.798
TC (mmol/L)	0.018	0.847
TG (mmol/L)	0.044	0.647
GLU (mmol/L)	-0.052	0.593
Ca (mmol/L)	-0.048	0.618
P (mmol/L)	0.101	0.294
BMD (g/cm^2)	0.608	< 0.001
Ferritin (ng/mL)	-0.478	< 0.001

Abbreviations: ALT, Alanine transaminase; ALP, Alkaline phosphatase; AST, Aspartate Transferase; BMD, Bone mineral density; BMI, Body mass index; BUN, Blood urea nitrogen; Ca, Calcium; Cr, Creatinine; GLU, glucose; P, Phosphorus; TC, Total cholesterol; TG, Triglyceride; PMOP, Postmenopausal osteoporosis

reporter assays, which showed a significant reduction in luciferase activity in the WT-PTEN group upon treatment with miR-106a-5p mimics, while no change was observed in the MT-PTEN group (Fig. 2B, $P<0.05$). PTEN expression was elevated in PMOP patients, and a similar increase in FAC-treated cells (Fig. 2C-D, $P<0.05$). Moreover, PTEN expression was negatively correlated with miR-106a-5p levels (Fig. 2E, $P<0.05$). The relative protein level of PTEN was decreased after treatment with miR-mimic (Fig. 2F, $P<0.05$).

miR-106a-5p promotes the osteogenic by regulating PTEN under iron accumulation

The role of the miR-106a-5p/PTEN axis in osteogenesis under conditions of iron accumulation was further investigated. FAC-treated decreased the osteogenic-related gene and cell proliferation and increased cell apoptosis (suppl Fig. 1). To investigate the regulatory mechanism, transfection experiments were performed in osteoblasts. Transfection efficiency was confirmed, showing that miR-106a-5p levels increased and PTEN expression decreased following miR-106a-5p mimic transfection under FAC treatment (Fig. 3A, $P<0.05$). Co-transfection of miR-mimic and a PTEN overexpression plasmid reversed the miR-106a-5p upregulation, with PTEN levels increasing accordingly (Fig. 3B, $P<0.05$). Osteogenesis-related gene expression was also elevated following miR-106a-5p mimic transfection, but this effect was reversed when PTEN was overexpressed in the same

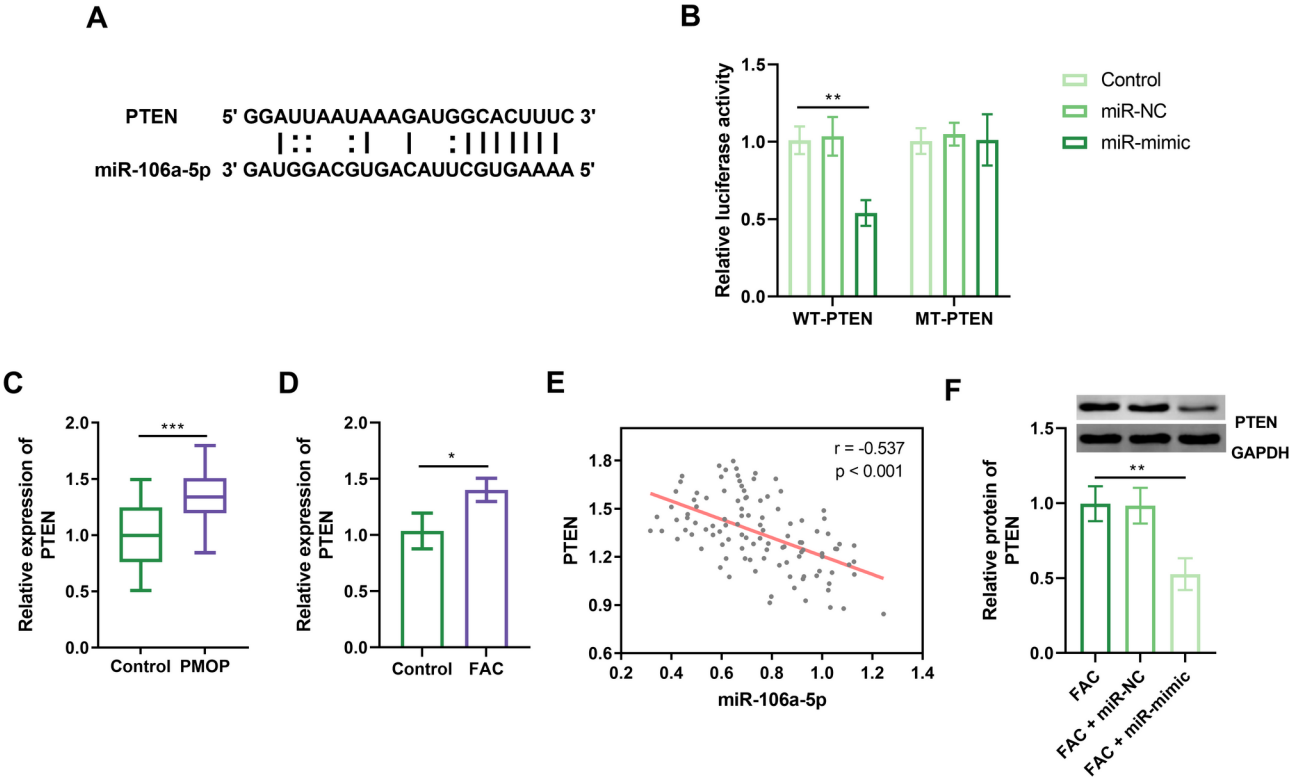

Fig. 2 miR-106a-5p directly targeted PTEN. (**A**) Predicted binding sites between miR-106a-5p and PTEN. (**B**) Dual-luciferase assay verified the target relationship ($n=3$). (**C**) Expression levels of PTEN in control ($n=112$) and PMOP ($n=108$) groups. (**D**) Expression levels of PTEN in FAC-treated hFOB1.19 cells ($n=3$). (**E**) The Pearson correlation analysis between miR-106a-5p and PTEN ($n=108$). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. Control. FAC, ferric ammonium citrate; PMOP, postmenopausal osteoporosis

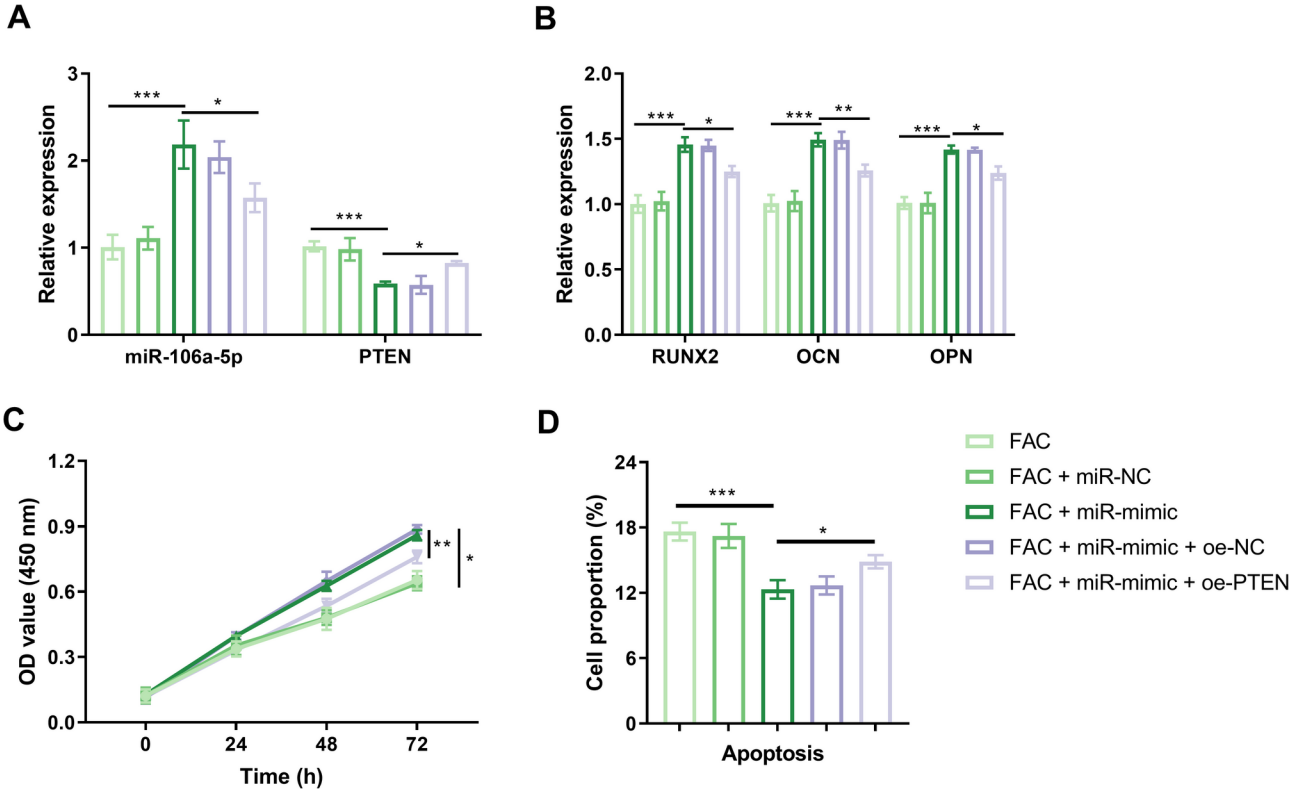

Fig. 3 miR-106a-5p enhanced osteogenic-related gene expression and cell proliferation by targeting PTEN ($n=3$). (**A**) Transfection efficiency of miR-mimic and oe-PTEN. (**B**, **C**) The levels of osteogenic-related genes (**B**) and cell proliferation (**C**) following transfection under FAC treatment. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. Control. FAC, ferric ammonium citrate

treatment group (Fig. 3B, $P<0.05$). Additionally, cell viability was significantly enhanced and cell apoptosis was reduced by miR-106a-5p mimic transfection under FAC conditions (Fig. 3C-D, $P<0.05$). However, overexpression of PTEN reversed the effects of miR-mimic on viability and apoptosis (Fig. 3C-D, $P<0.05$). These results suggest that under iron accumulation conditions, miR-106a-5p regulates osteogenesis by targeting PTEN.

Discussion

OP is a largely asymptomatic condition in its early stages, affecting a significant portion of the global population [2, 30]. Gender differences in prevalence are prominent, with OP particularly affecting postmenopausal women [7]. Iron metabolism plays a critical role in maintaining bone health. Under normal physiological conditions, iron is absorbed through the intestines, binds to transferrin in the bloodstream, and is utilized for cellular functions or stored in organs [31]. However, when the iron pool exceeds the binding capacity of transferrin, excess iron accumulates in tissues in the form of serum ferritin, including in bone tissue [32]. With aging—particularly in postmenopausal women—iron levels tend to rise as estrogen levels decline due to the cessation of menstruation [11, 33]. Recent research has further highlighted that iron accumulation is not only associated with the development of OP and brittle fractures but that its effects on bone metabolism are more pronounced in postmenopausal women compared to elderly men [34, 35]. These observations highlight the importance of investigating the role of iron overload in postmenopausal OP and understanding its contribution to impaired bone homeostasis. Serum ferritin, a primary iron storage protein, is widely used as a clinical indicator of iron status [36]. Considering that ferritin has been identified as an essential factor for OP [9], serum ferritin levels in the patients included were measured in this study. The results demonstrated that ferritin levels were significantly elevated in the PMOP group, corroborating previous findings that iron accumulation is a key factor in PMOP. This highlights the potential role of ferritin as a biomarker for OP and suggests that iron overload contributes to the pathophysiology of PMOP.

As research into the role of miRNAs in disease progresses, increasing evidence suggests that miRNAs could serve as valuable biomarkers or therapeutic targets, especially in bone metabolism-related diseases including OP [12, 37, 38]. In recent years, growing evidence has highlighted the role of miRNAs in the pathogenesis of OP. For example, miR-128-2-5p has been shown to inhibit

the progression of PMOP by reducing osteoblast adhesion [39], while miR-4534 is upregulated in OP and may have diagnostic value [40]. Furthermore, miRNA expression profiles identified through omics analyses have been strongly associated with osteoblast differentiation and alterations in BMD [41]. Our study revealed that miR-106a-5p is significantly downregulated in PMOP patients and its expression is closely correlated with BMD. The ROC curve analysis further demonstrated that miR-106a-5p possesses notable diagnostic value, suggesting its potential as a biomarker for PMOP. miR-106a-5p has previously been implicated in various diseases; for example, it is involved in the pathogenesis of rheumatoid arthritis via the angiogenesis pathway [42], and it is downregulated in cisplatin-resistant osteosarcoma cells [43]. It is reported that the dysregulation of miR-106a-5p was related to the progression and prognosis of osteosarcoma [24]. In adolescent idiopathic scoliosis and osteopenia, miR-106a-5p may play a key role in mesenchymal stem cell differentiation [44]. Additionally, miR-106a-5p has been shown to target SLC2A3, a gene associated with ferroptosis [45]. Recent studies also highlight its role in intercellular communication between osteoblasts and osteoclasts, influencing osteoblast differentiation [22]. The present findings further confirm that miR-106a-5p is not only associated with bone density but also negatively correlated with ferritin, a marker of iron accumulation. Moreover, in an iron overload cell model, miR-106a-5p expression was significantly downregulated following FAC treatment, suggesting its involvement in the pathogenesis of OP under iron overload conditions.

To elucidate the mechanism through which miR-106a-5p exerts its effects on PMOP, the downstream target genes were investigated. PTEN, a key regulator of ferroptosis in PMOP [28], was identified as a target of miR-106a-5p. Previous studies have demonstrated that PTEN regulates iron pool content and tumor growth in colorectal cancer [46]. Moreover, miR-29b-3p promotes spinal cord injury repair by targeting PTEN [47]. In this study, the interaction between miR-106a-5p and PTEN was validated through gene expression analysis, correlation analysis, and dual-luciferase assays. It is reported that PTEN was associated with iron metabolism. In chondrocytes, PTEN promotes osteoarthritis progression via the ferroptosis pathway [48]. PTEN was identified as a novel gene associated with ferroptosis in pediatric sepsis [49]. In the iron accumulation cell model, miR-106a-5p positively regulated osteoblast function by targeting PTEN. In the iron accumulation cell model, miR-106a-5p positively regulated osteoblast function by targeting PTEN. Osteoblast proliferation is a critical first step in bone formation [50]. Additionally, abnormal osteoblast apoptosis leads to a reduced rate of bone formation [51, 52]. RUNX2, a transcription factor crucial for osteoblast differentiation, regulates downstream genes such as osteopontin (OPN) and osteocalcin (OCN), which are essential for bone mineral deposition and remodeling [53, 54]. The upregulating miR-106a-5p enhanced the expression of osteoblast-related genes, promoted cell proliferation, and decreased cell apoptosis. However, when PTEN was overexpressed, this effect was reversed. These results indicate that under iron overload conditions, miR-106a-5p could promote osteogenesis by targeting PTEN.

This study has some limitations. As a preliminary investigation, we primarily focused on evaluating the diagnostic potential of miR-106a-5p and exploring its regulatory role in osteoporosis. These findings offer preliminary evidence for the role of the miR-106a-5p/PTEN axis in iron-induced dysregulation of bone metabolism and we acknowledge that the precise molecular mechanisms remain to be fully elucidated, particularly those related to osteoblast differentiation and mineralization. Future studies should incorporate additional assays such as Alizarin Red Staining and alkaline phosphatase activity to further clarify these effects. This study employed the hFOB1.19 human osteoblast as an in vitro model. This model is useful for studying osteoblast biology. However, it does not fully replicate the physiological environment of bone marrow-derived mesenchymal stem cells (BMSCs), which are directly involved in bone formation and remodeling. Future studies incorporating primary human BMSCs will be essential to validate the findings in a more physiologically relevant context and further evaluate the role of miR-106a-5p in osteogenic differentiation and mineralization [20]. Although miR-106a-5p demonstrated promising diagnostic value, we did not assess its diagnostic performance in combination with other established PMOP biomarkers. This may limit the clinical translational potential of our findings. Future studies should include joint ROC curve analyses incorporating multiple biomarkers to evaluate whether miR-106a-5p improves diagnostic sensitivity and specificity when used in combination. This study used Pearson correlation and t-tests to assess associations between miR-106a-5p, BMD, and ferritin. However, potential confounding variables such as age and BMI were not adjusted for in multivariate regression models, which may limit the ability to draw definitive conclusions about independent associations. Future studies with larger sample sizes should incorporate multivariate analyses to better account for these factors and validate the current findings. While our findings suggest that miR-106a-5p holds promise as a diagnostic biomarker, further in vivo validation is required. Additionally, future studies comparing its diagnostic performance with other established PMOP-related biomarkers will be essential to clarify its unique advantages and clinical utility. This study proposes a potential

regulatory relationship between iron accumulation and the miR-106a-5p/PTEN axis, the individual steps of this pathway have not yet been conclusively linked through direct mechanistic evidence. In particular, whether iron overload directly downregulates miR-106a-5p or acts through intermediate factors remains unclear. Further studies using detailed mechanistic experiments and broader data validation are required to confirm these interactions.

Many diseases are linked to problems in specific signaling pathways [55]. Mechanistically, while we confirmed that miR-106a-5p targets PTEN to regulate osteoblast proliferation, the downstream signaling pathways of PTEN require further investigation. AKT, a key protein kinase, plays a critical role in regulating cell growth, proliferation, and survival, and PTEN is well-established as its negative regulator [56, 57]. In the context of osteoblast differentiation, a study has shown that inhibition of PTEN can enhance AKT activation, subsequently stimulating the NF-κB signaling pathway and ultimately suppressing osteoblast differentiation [58]. In addition, reducing PTEN ubiquitination has been shown to inhibit RANKL-induced activation of the NF-κB pathway, thereby suppressing the expression of osteoclastogenesis-related genes [59]. The relationship between NF-κB signaling and iron overload is both close and complex. Animal experiments have demonstrated that inhibition of NF-κB signaling can alleviate osteoarthritis progression caused by iron accumulation [60]. Moreover, numerous studies have reported NF-κB activation in osteoporosis [61, 62]. Given PTEN's role as an upstream inhibitor of the AKT pathway, it is plausible that miR-106a-5p may regulate osteogenesis by suppressing PTEN and modulating the AKT/NF-κB signaling. Although this regulatory link was not directly examined in our current study, it presents a compelling direction for future research. Specifically, evaluating AKT phosphorylation levels and downstream effectors such as NF-κB in the context of miR-106a-5p/PTEN modulation will help elucidate this pathway. Future experiments will further explore these mechanisms to provide a more comprehensive understanding of PMOP.

Conclusion

In conclusion, miR-106a-5p shows diagnostic potential for PMOP and is associated with iron accumulation. miR-106a-5p holds promise as a biomarker for PMOP. It appears to promote osteogenesis by regulating PTEN under conditions of iron overload. Our study provides a foundational reference for further research and potential therapeutic strategies of PMOP.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13018-025-05872-3.

Supplementary Material 1

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Not applicable.

Author contributions
Xiangjie Liu: Conceptualization, Data curation, Investigation, Resources, Software, Supervision, Writing - original draft. Xiaogang Zhang: Formal analysis, Investigation, Software, Visualization, Writing - review & editing. Meini Cen: Formal analysis, Methodology, Project administration, Validation, Visualization, Writing - review & editing.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All volunteers were able to independently provide consent and signed informed consent forms. Approval was obtained from the ethics committee of The First Affiliated Hospital of Chengdu Medical College. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Conflict of interest
No conflict of interest has been declared by the authors.

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