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ZFP36, an RNA-binding protein promotes hBMSCs osteogenic differentiation via binding with JUN

Hairong Su^{1†}, Linyuan Liang¹, Junling Wang², Xiaolu Yuan^{1*} and Binxiu Zhao^{1*}

Abstract

Osteoporosis (OP) is a metabolic bone disease characterized by progressive decline of bone mass and bone quality, leading to bone fragility and an increased risk of fracture. The osteogenic differentiation of bone mesenchymal stem cells (BMSCs) is crucial to maintain the balance of osteoblast and osteoclast. Bioinformatics prediction indicates that ZFP36 ring finger protein (ZFP36), an RNA-binding protein, is a potential target of OP. Herein, we sought to probe the regulatory role and mechanisms of ZFP36 in the progression of OP. Overexpression of ZFP36 enhanced osteoblast viability, differentiation and mineralization of human BMSCs (hBMSCs). RNA immunoprecipitation qPCR (RIP-qPCR) assays demonstrated that ZFP36 could inhibit the translation of JUN, which was also verified with dual luciferase reporter gene assay. Furthermore, administration with T-5224, a transcription factor c-Fos/activator protein (AP)-1 inhibitor, which specifically inhibits the DNA binding activity of c-Fos/JUN, abolished the effect of ZFP36 knockdown on the behaviors of hBMSCs, suggesting that ZFP36 might promotes osteogenic differentiation through regulating JUN. These findings provide insights into the progression and a potential therapeutic target of OP.

Keywords Osteoporosis, ZFP36 ring finger protein, JUN, Osteogenic differentiation

Introduction

Osteoporosis (OP) is a systemic metabolic bone disease caused by the decrease of bone density and bone quality, the destruction of bone microstructure, and the increase of bone fragility [1]. The incidence of OP is increasing rapidly and has huge financial and emotional costs [2]. At present, OP is mainly treated with drug intervention

or hormone therapy, which directly stimulates bone formation to increase bone mass. However, long-term drug treatment will produce serious side effects [3]. The occurrence of OP is closely related to the imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption, and the osteogenic differentiation and bone remodeling of bone mesenchymal stem cells (BMSCs) is crucial to maintain the balance [4, 5]. Thus, we sought to identify pivotal molecules and mechanisms involved in the osteogenic differentiation of BMSCs.

ZFP36 ring finger protein (ZFP36), an RNA-binding protein, regulates a variety of post-transcriptional processes via destabilizing several cytoplasmic AU-rich element (ARE)-containing mRNA transcripts and attenuating protein synthesis [6]. ZAP36 is involved in

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several processes, including cellular response to cytokine stimulus, cellular response to growth factor stimulus, and regulation of gene expression. It also plays a role in anti-inflammatory responses, anti-oxidative stress, proliferation, differentiation and apoptosis, which indicating ZFP36 may be a potent therapeutic target of several diseases, such as cancer and rheumatoid arthritis [7]. ZFP36L1, a key member of the ZFP36 family, exerts a crucial regulatory function in monocyte/macrophage differentiation by targeting CDK6 mRNA for degradation. It also acts as an anti-inflammatory factor by destabilizing mRNA transcripts of pro-inflammatory cytokines, such as TNF- α , in macrophages. In the context of osteoarthritis, ZFP36L1 upregulation in chondrocytes is linked to the modulation of HSP70 family mRNA stability, which confers protection against chondrocyte apoptosis, underscoring its role in inflammatory response modulation. Within the scope of osteoporosis, where the balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption is critical, our research delves into the impact of ZFP36 on the osteogenic differentiation and bone remodeling of bone mesenchymal stem cells (BMSCs). Of interest, the expression of ZFP36 is increased at high bone mineral density (BMD) patients, a reduction of BMD is characterized in OP patients, which suggesting ZFP36 maybe a potential role of OP [8].

JUN has been demonstrated in driving bone formation and accelerating bone growth which offering a novel therapeutic approach to OP [9]. The expression of c-Jun was remarkably upregulated during MSC osteogenic differentiation, while knocking down c-Jun significantly reduced ALP activity and osteoblastic marker expression, indicating JUN as a key transcription factor involved in the regulation of the osteogenic differentiation [10]. However, it remains unclear whether it is regulated by ZFP36 in osteoblasts.

Here, we examined ZFP36 differential expression in OP patients and overexpressing of ZFP36 promoted the osteoblastic differentiation of human hBMSCs. Mechanistically, ZFP36 inhibited the translation of JUN by binding to the 3'UTR of JUN mRNA via RIP-qPCR analysis. Moreover, the attenuated effect of ZFP36 knock-down on the osteogenic differentiation of hBMSCs was reversed by T-5224, an inhibitor of JUN. These findings identify ZFP36 as a promising target for OP.

Methods and materials

Data extraction

The microarray datasets (GSE2208 and GSE56815) were downloaded from GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). These datasets included 50 females with high bone mineral density (BMD) and 49 with low BMD which were associated with osteoporosis. These datasets were produced by an Affymetrix Human Genome U133A

Array platform. All microarrays were downloaded with $\log_2$ -transformed and normalized.

Differential Expression Genes (DEGs) analysis

DEGs were identified in the two datasets and analyzed with the R software “limma” package. Genes with $|\log_2$ fold change| > 1.0, False discovery rate value < 0.05 and P value < 0.05 were defined as DEGs. The heatmap and volcano map of the DEGs was used by R software “Heatmap” and “ggplot2” package, respectively. The overlap intersection part of the top 50 up-regulated DEGs or down-regulated DEGs was analyzed by the Venn Diagram online tools (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

Functional enrichment analysis

The DEGs were imported into the David database (<https://david.ncifcrf.gov/>) for Gene Ontology (GO, including cellular component (CC), molecular function (MF), and biological process (BP)) enrichment analysis ($P < 0.05$), and the results were visualized by GraphPad Prism 8.0.2. The R software “clusterProfiler” package was used to perform KEGG pathway enrichment analysis of the DEGs with $P < 0.05$ and the R software “ggplot2” package was used to visualize the results.

Protein-Protein Interaction (PPI) networks

The STRING database (<https://string-db.org/>) was used to construct the protein protein interaction network (PPI) between co-expressed DEGs and visualized by Cytoscape (version 3.9.1). The network characteristics of each node were calculated and analyzed by “Degree” algorithm in cytoHubba plug-in.

Cell culture and osteogenic differentiation

This study was approved by the ethics committee of the Medical Ethical Committee of the Maoming People's Hospital. Primary human bone marrow-derived mesenchymal stem cells (hBMSCs) were isolated from bone marrow and separated by density gradient centrifugation and cultured in DMEM culture medium (Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA), and 1% penicillin–streptomycin under 5% CO₂ at 37 °C. The non-adherent cells were removed with culture medium changed every three days. For osteogenic differentiation, the hBMSCs were induced in osteogenic induction medium composed of DMEM, 10% FBS, 100 nM dexamethasone, 10 mM β -glycerophosphate, and 50 μ M ascorbic acid-2 phosphate. The differentiation capacity and calcium deposit were measured by alkaline phosphatase (ALP) staining and Alizarin Red Staining according to the manufacturer's protocol, respectively.

Cell proliferation assay

To assess the proliferation of hBMSCs, 5,000 cells/well were seeded into 96-well plates with growth medium for 0 h, 24 h, 48 h, and 72 h. Then 10 μ l Cell Counting Kit-8 (CKK-8; Dojindo, Japan) reagent was added into the cells for 1 h at 37 °C. The absorbance at 450 nm was measured by a microplate reader (BioTek, USA).

Lentiviral packaging and cell infection

The cDNA encoding human ZFP36 was inserted into a lentiviral vector as ZFP36-overexpression (ZFP36-OE). The shRNA targeted ZFP36 mRNA (5' GACGGAACTCTGTACAAGTT 3') was constructed into a shRNA lentiviral vector as ZFP36-knockdown (ZFP36-sh). The recombinant lentivirus was produced in 293T cells co-transfected with the recombinant plasmids using PEI reagent according to the protocol. The supernatant was collected after transfection for 48 h. hBMSCs were incubated with lentivirus in growth medium with 2 μ g/ml polybrene (Sigma-Aldrich, USA) and 1 μ g/mL puromycin for 2 weeks to select the positive cells.

Real-time qPCR analysis

Total RNA was extracted with using an RNA isolation kit (Tiangen Biotech, China) and synthesized to cDNA by a reverse transcription kit (Takara, Japan) according to the manufacturer's protocol. The levels of mRNAs were determined by SYBR Green Master Mix using a Step One Real-Time PCR system (Applied Biosystems Inc., UK). The concentrations of target genes were measured using the $2^{-\Delta\Delta C_t}$ method and GAPDH was used as a house-keeping gene. All primers (ZFP36 Forward: 5' GACTGAGCTATGTCGGACCTT 3', ZFP36 Reverse: 5' GAGTTC CGTCTTGTTATTTGGGG 3'; JUN Forward: 5' TCCAAGTGCCGAAAAGGAAG 3'; JUN Reverse: 5' CGAGTTCTGAGCTTTCAAGGT 3') used in this experiment were synthesized by Sangon Biotech and are listed below:

Western blotting

Cells were harvested and lysed with RIPA lysis buffer containing protease inhibitors. The concentrations of the lysed protein were determined using a BCA protein assay kit (Thermo Fisher, USA). Equal amounts of proteins were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA). After blocking with 5% BSA for 1 h at room temperature, the membranes were incubated with primary antibodies (1:1000, all purchased from Abcam) at 4 °C overnight and secondary antibodies for 45 min at room temperature. Finally, signal intensity was measured using enhanced chemiluminescence blotting reagents (Millipore, USA) on a Bio-Rad detection system (Bio-Rad, USA).

RIP RT-qPCR analyses

Cells were washed with PBS and lysed with lysis buffer according to the manufacturer's instructions. For immunoprecipitation, the supernatant was incubated overnight at 4 °C with 5 μ g ZFP36 antibody and control IgG-antibody. The immunoprecipitates were further incubated with protein A/G Dynabeads (Thermo Scientific) for 2 h at 4 °C. RNA was extracted with using an RNA isolation kit (Tiangen Biotech, China), then the cDNA library was built and assessed using RIP-seq and RT-qPCR following the manufacturer's instructions. The concentrations of target genes were measured using the $2^{-\Delta\Delta C_t}$ method and GAPDH was used as an internal control.

Dual luciferase reporter gene assay

The ZFP36 3'-UTR (ZFP36-OE) and truncate (ZFP36-OE-Cut, PF00642+PF14608) and JUN 3'-UTR (JUN-WT) sequences were cloned into pMIR-reporter. The correctly sequenced luciferase reporter plasmids ZFP36-OE and ZFP36-OE-Cut were co-transfected with JUN-WT into HEK-293T cells for 48 h. Then the cells were lysed and the luciferase activities were measured by a Dual-Glo Luciferase Assay Kit (Promega, USA).

Statistical analysis

GraphPad Prism 8.0 software (La Jolla, USA) was used to analyze the data. The measurement data were expressed as mean $\pm$ SD. The unpaired t test was used to compare the data between two groups. The one-way analysis of variance (ANOVA) was utilized to analyze the data among multiple groups. $p < 0.05$ was considered statistically significant.

Results

ZFP36 was differentially expressed in osteoporosis

The expression profiles by the array of GSE2208 and GSE56815 were retrieved from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) to screen the differentially expressed genes (DEGs) of OP using the R software package. There were 748 down-regulated genes and 312 up-regulated genes in GSE2208, and 904 down-regulated genes and 1718 up-regulated genes in GSE56815, respectively. Then the overlap between different gene sets were represented by Venn diagrams using the Venn Diagram online tools (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). A total of 44 DEGs were co-expressed, including 32 up-regulated genes and 12 down-regulated genes (Fig. 1A and B). The results of functional enrichment analysis were presented in Fig. 1C and D. The KEGG functional enrichment analysis revealed that these genes were related to osteoclast differentiation (Fig. 1C). The GO analysis revealed that transcription factor activity and cell proliferation were enriched (Fig. 1D). To understand the interactions between these DEGs, a PPI network was

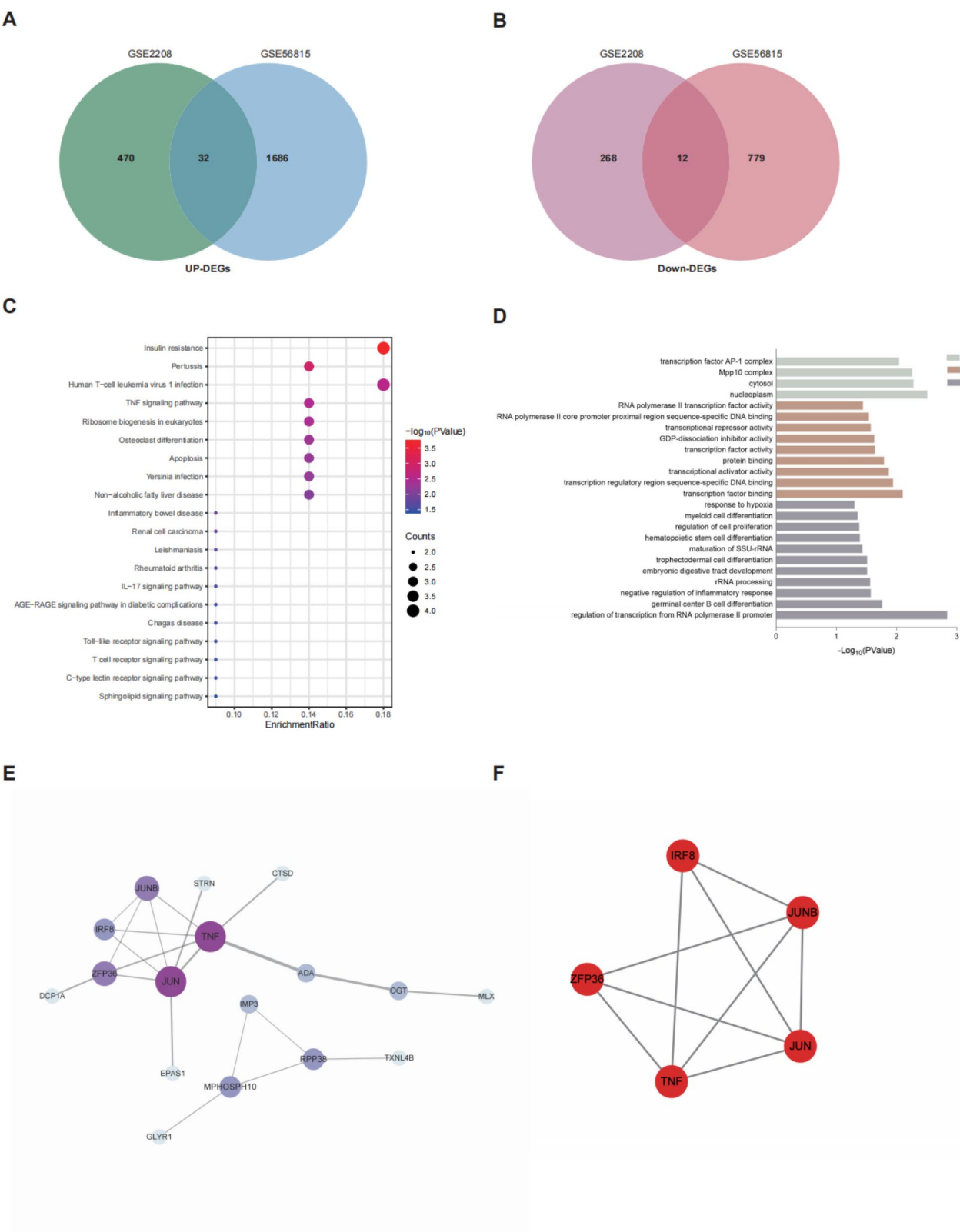


Fig. 1 ZFP36 was differentially expressed in osteoporosis. **(A)** The overlap of upregulated-DEGs was represented by Venn diagrams between GSE2208 and GSE56815. **(B)** The overlap of downregulated-DEGs was represented by Venn diagrams between GSE2208 and GSE56815. **(C)** The KEGG functional enrichment analysis of these DEGs. **(D)** The GO analysis of these DEGs. **(E)** The PPI analysis of these DEGs. **(F)** Cluster analysis of the 5 hub genes performed using the MCODE plugin

constructed using STRING software and the results were imported into the Cytoscape software for co-expressing DEGs. The central hub genes were identified depending on the color, degree, maximum of the edge. The PPI network has 17 nodes and 21 edges, and genes like ZFP36, JUN, JUNB, TNF and IRF8 were served as central genes (Fig. 1E). In addition, cluster analysis of these hub genes was also performed using the MCODE plugin and found that ZFP36, JUN, JUNB, TNF and IRF8 were positively correlated (Fig. 1F). Since ZFP36, an RNA-binding gene, was demonstrated regulating inflammation and proliferation via regulating different target mRNAs. However, no previous study has examined whether ZFP36 involved in OP pathogenesis, we then investigated the possible association of ZFP36 in OP.

ZFP36 improve osteoblast proliferation, differentiation, and mineralization

Then the effects of ZFP36 on the proliferation, differentiation, and mineralization of hBMSCs were explored by CCK8 assay, ALP staining, Alizarin Red Staining, qPCR and Western blotting. hBMSCs have been successfully characterized, exhibiting the phenotypic markers (including CD105, CD106, and CD34) indicative of their

identity (Supplemental Fig. 2A). Furthermore, we have established stable cell lines with either overexpression or knockdown of the ZFP36 gene, which have also been validated by qPCR and WB (Supplemental Fig. 2B-2D). The CCK8 assay revealed a remarkably elevated proliferation of hBMSCs in the ZFP36 overexpression group (ZFP36-OE) than in the negative control with a time-dependent manner, while the proliferation was reduced in the ZFP36-knockdown (ZFP36-sh) group (Fig. 2A). Mineralized nodule content by Alizarin Red Staining (Fig. 2B) and ALP staining (Fig. 2C) were higher in the ZFP36 overexpression group cells than in the negative control, which were also decreased after the ZFP36 was knockdown. In addition, the protein level of osteoblastic markers, including ALP, OCN, OPN and Runx2, was measured by western blotting. The bone differentiation markers were markedly elevated after the ZFP36 was overexpressed, while decreased in the ZFP36-sh group, compared with the negative control (Fig. 2D). These results imply that ZFP36 improve osteoblast proliferation, differentiation, and mineralization of hBMSCs in vitro.

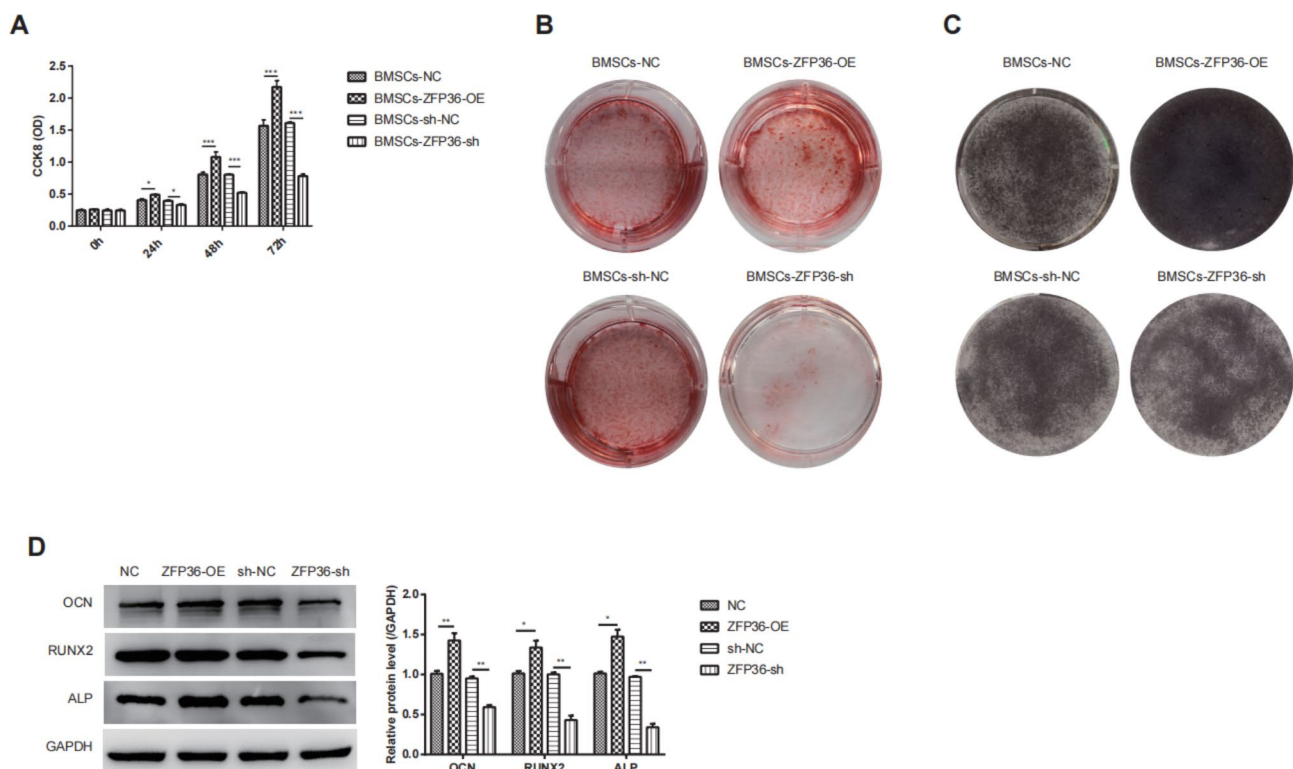


Fig. 2 ZFP36 improve osteoblast proliferation, differentiation, and mineralization. **(A)** Cell viability of BMSCs transfected with ZFP36 overexpression, ZFP36 knockdown, and negative controls. **(B)** Mineralized nodule content by Alizarin Red Staining of BMSCs transfected with ZFP36 overexpression, ZFP36 knockdown, and negative controls. **(C)** ALP staining of BMSCs transfected with ZFP36 overexpression, ZFP36 knockdown, and negative controls. **(D)** The protein level of osteoblastic markers in BMSCs transfected with ZFP36 overexpression, ZFP36 knockdown, and negative controls. Data represent means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$

ZFP36 bound to the 3'UTR of JUN mRNA

We then aimed to investigate the the molecular mechanisms underlying ZFP36-mediated regulation of hBM-SCs with an algorithm to estimate the binding propensity of protein-RNA pairs by catRAPID omics v2.0 and verified with RIP assays. The protein and functional region targeted by ZNF36 were analyzed. As show in Fig. 3A, our findings revealed a significant enrichment of binding domains spanning the 104–168 amino acid. The analysis identified PF14608 (with a predicted RBD from amino acids 108 to 129) and PF00642 (with RBDs starting at amino acids 104 to 130 and 142 to 168) as the principal binding sites. Following this, we conducted an

intersection analysis between the mRNAs predicted to bind to these domains and the hub genes identified in Fig. 1E. This intersection analysis revealed that the JUN gene was a prominent candidate. Then the WB analysis indicated that RNAs or proteins were effectively pulled down by ZFP36 (Fig. 3B). In addition, RIP-qPCR data revealed that JUN mRNA was significantly enriched in cells incubated with anti-ZFP36 antibody with double repetition (Fig. 3C). We then determine the binding region of ZFP36 to JUN mRNA by dual luciferase reporter gene assay. The ZFP36 overexpression sequence has been designated as ZFP36-WT. Concurrently, the sequence spanning amino acids 104–168, which is

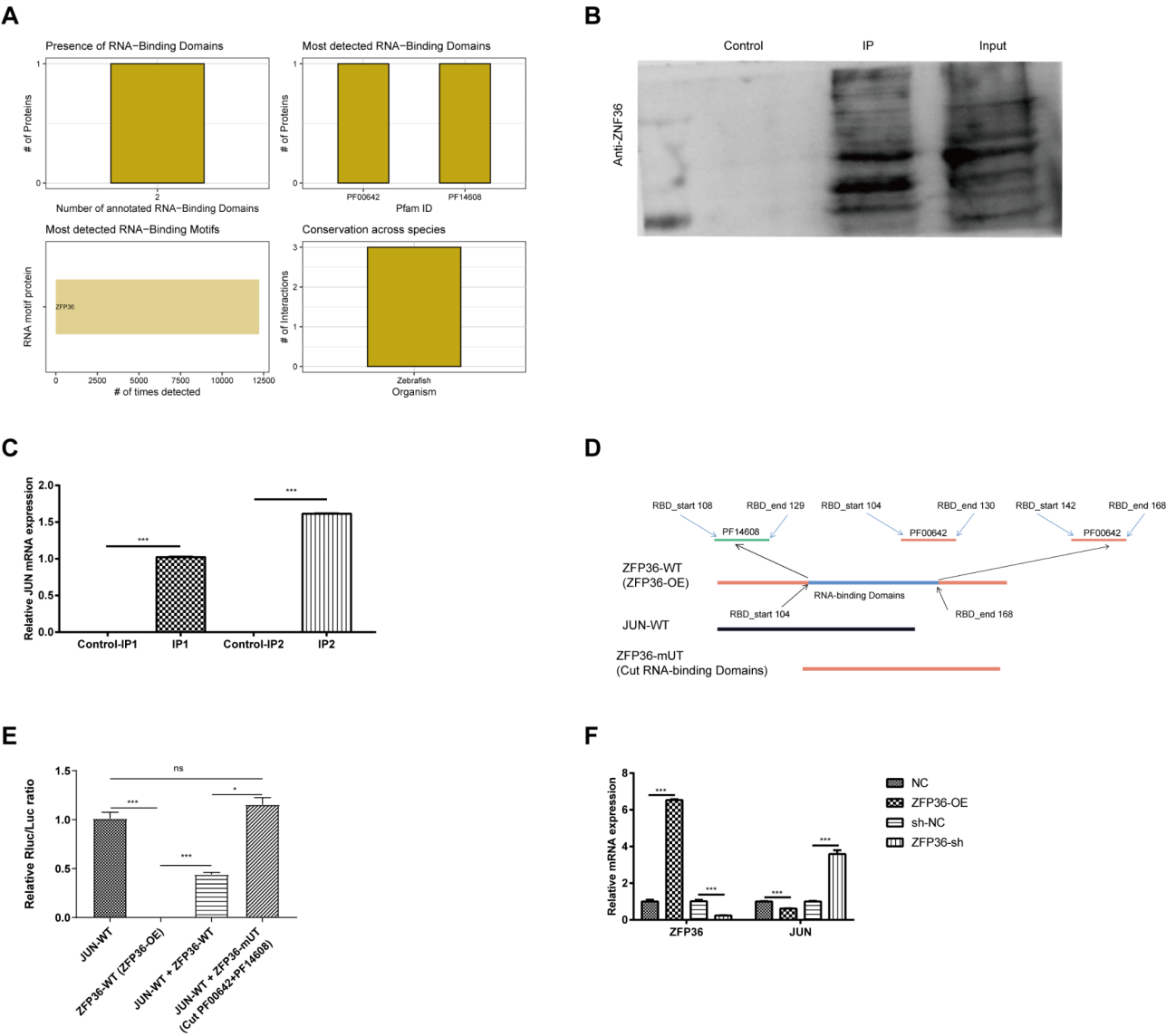


Fig. 3 ZFP36 bound to the 3'UTR of JUN mRNA. **(A)** The protein and functional region targeted by ZNF36 were analyzed by catRAPID omics v2.0. **(B)** The binding efficiency of ZFP36 to other proteins was detected by western blotting. **(C)** A detection of binding affinity of ZFP36 to JUN mRNA by RIP assay. **(D)** Plasmid construction of ZFP36 OE and ZFP38 mut. **(E)** Binding affinity of JUN to ZFP36 in different segments detected by dual luciferase reporter gene assay. **(F)** The mRNA expression of JUN and ZFP36 in BMSCs transfected with ZFP36 overexpression, ZFP36 knockdown, and negative controls. Data represent means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$

recognized as the binding domain, has been excised and is referred to as ZFP36-MUT. The JUN sequence was constructed within the pMIR-GLO dual-luciferase vector, while the ZFP36 and analogous sequences were constructed within the pCDNA3.1 vector. For the purpose of elucidating the interaction between these sequences, 293T cells, were selected as the utility cells for co-transfection. The dual-luciferase assay was employed to determine the binding affinity between the ZFP36-WT or its mutant variant ZFP36-MUT and the JUN sequence. The luciferase reporter assay results revealed that overexpression of wild-type ZFP36 lead to a significant decrease of the luciferase activity compared to JUN-WT transfection, an effect that was reversed upon the deletion of the RBD (Fig. 3D and E). This observation underscores the significance of the RBD in the interaction between ZFP36 and JUN. Additionally, the results of qPCR assay revealed that JUN mRNA expression was decreased in ZFP36-OE group, while ZFP36 knockdown upregulated JUN (Fig. 3F). Taken together, ZFP36 could bind to the 3'UTR of JUN mRNA.

ZFP36 enhanced the osteogenic differentiation by regulating JUN

Next, we explore whether ZFP36 regulates osteogenic differentiation through regulation of JUN. After infection of shZFP36 in hBMSCs, the cell viability was decreased, whereas the proliferation of hBMSCs was augmented in response to additional treatment of T-5224, an inhibitor of c-Fos/JUN (Fig. 4A). Moreover, ALP activity (Fig. 4B) and Alizarin red S staining assay (Fig. 4C) was reduced in response to ZFP36 knockdown, which was negated in the additional treatment with T-5224. Furthermore, the expression of RUNX2, OCN, and ALP was diminished in ZFP36-sh group, whereas the expression was counteracted upon its combination with T-5224 (Fig. 4D). To sum up, JUN inhibition by T-5224 reversed the promotion effect of ZFP36 knockdown on the osteogenic differentiation of hBMSCs, which indicated that ZFP36 could enhance the osteogenic differentiation by regulating JUN.

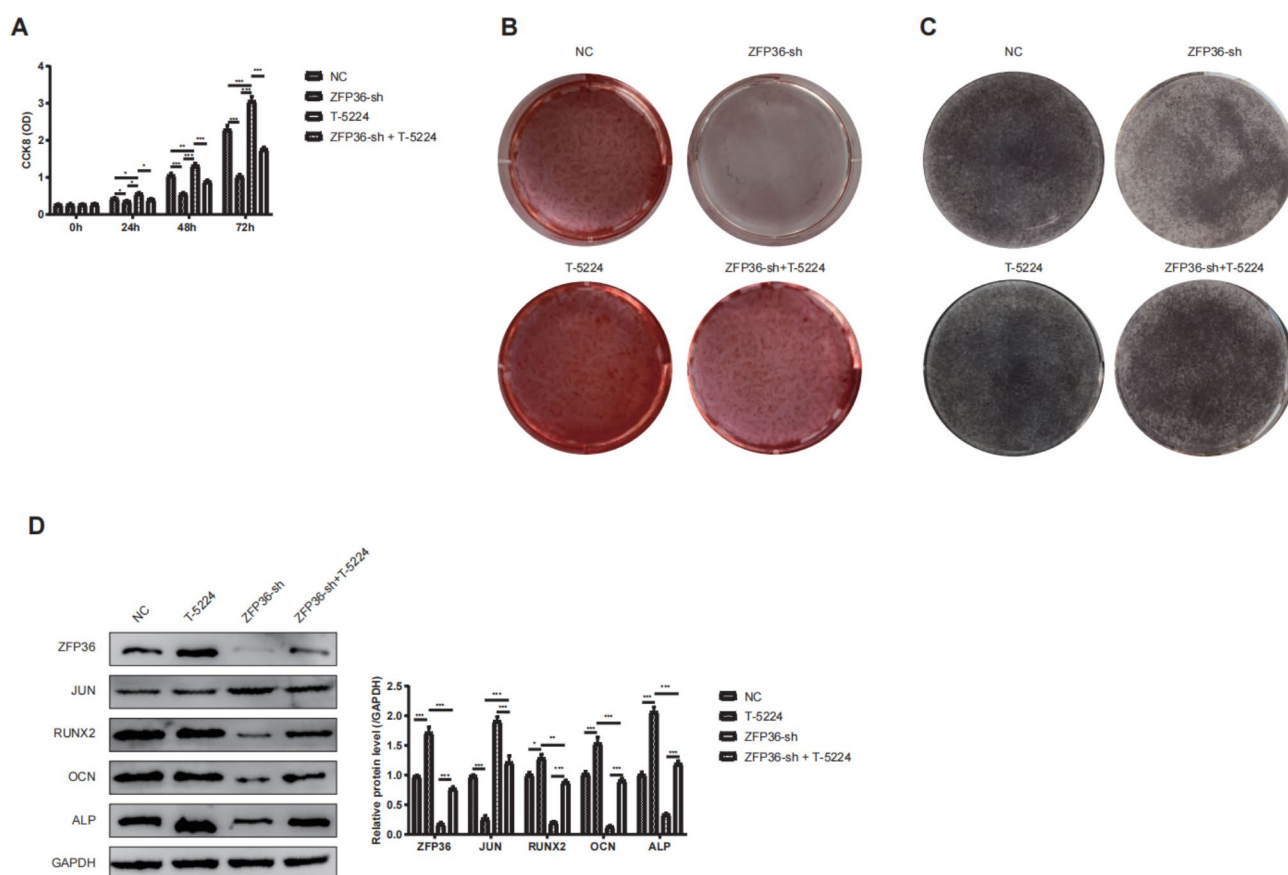


Fig. 4 ZFP36 enhanced the osteogenic differentiation by regulating JUN. **(A)** Cell viability of BMSCs in response to ZFP36 knockdown alone or in combination with T-5224, a JUN specific inhibitor. **(B)** Mineralized nodule content by Alizarin Red Staining of BMSCs in response to ZFP36 knockdown alone or in combination with T-5224. **(C)** ALP staining of BMSCs in response to ZFP36 knockdown alone or in combination with T-5224. **(D)** The protein level of osteoblastic markers in BMSCs in response to ZFP36 knockdown alone or in combination with T-5224. Data represent means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$

Discussion

Osteoporosis is characterized by reduced bone mass and microstructural damage that is resulted from the injury of the process during osteogenic differentiation and bone remodeling [11]. However, the pathogenesis of OP is incompletely understood. A better understanding of its pathogenesis contributes to developing novel and efficient therapeutic approaches to OP. Here in this study, according to bioinformatics prediction and in vitro experiments, we speculate that the RNA-binding protein ZFP36 may regulate the development of OP by targeting JUN.

We integrated multiple OP microarray datasets to identify DEGs in the progression of OP and found that ZFP36, an RNA-binding protein, is a potential target of OP. Previous studies have shown that ALP staining, Alizarin Red Staining, and the expression of RUNX2, OCN and ALP are well-established markers of osteogenic differentiation [12, 13]. Here, the results showed that overexpressing of ZFP36 could result in enhanced ALP activity and increased mineralized nodule content along with expression of osteogenic differentiation markers, which were all reduced by silencing of ZFP36. Therefore, ZFP36 may have the potential to serve as a therapeutic target against OP.

RNA-binding proteins are a class of targeted binding to specific RNAs, playing important roles in RNA stability and RNA translation of post-translational regulatory processes [14, 15]. The targeting function of RNA binding proteins is based on adenylate and uridylate rich elements (AREs) in the 3'-UTR of mRNA [6, 16]. The relationship between RNA-binding proteins and transcription factors of BMSCs in the progress of OP has not been fully explored [17, 18]. The ZFP36 family is a group of highly conserved RNA binding proteins with CCCH-type RNA binding domains, including ZFP36, ZFP36 ring finger protein-like 1 (ZFP36L1), ZFP36 ring finger protein-like 2 (ZFP36L2), and ZFP36 ring finger protein-like 3 (ZFP36L3) found in rodent placental tissue [19, 20]. The most important biological function of ZFP36 protein is post-transcriptional regulation. Through the tandem zinc finger structure and the ARE of the 3'-UTR of the target mRNA, ZFP36 promotes the deadenylation of target mRNAs to remove the polyadenylate tail structure, thereby degrading the target mRNA and regulating the expression of inflammatory factors and transcription factors. ZFP36 protein is involved in numerous biological processes, such as cell cycle transition, cell differentiation and anti-inflammation [21–23].

Mechanistically, both the luciferase reporter assay and qPCR assay have demonstrated that ZFP36 downregulates the expression of JUN by binding to the 3'UTR of the JUN mRNA, thereby inhibiting its translation. In vitro study also found that the role of ZFP36 in inducing

osteogenic differentiation was reversed by T-5224, the inhibitor of JUN. A previous study has elucidated that JUN promote the osteogenic differentiation of osteoblasts. Taken together, this study firstly represents the post-transcriptional regulation of JUN by ZFP36 in hBMSCs and may have importance in regulating the progression of OP.

In conclusion, ZFP36 facilitated osteogenic differentiation via binding to the 3'UTR of JUN to inhibit its expression. This study helps us to deepen the understanding of RNA-binding protein ZFP36 in the pathogenesis of OP. These findings suggest a novel therapeutic target for this disease and further clinical trials are needed for the development of ZFP36-based therapeutic agents.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13018-024-05232-7>.

Supplemental Fig. 1. Identification of DEGs in osteoporosis. (A) The top 40 DEGs from the GSE2208 dataset. (B) The top 40 DEGs from the GSE56815 dataset. (C) Volcano plot analysis for the GSE2208 dataset. (D) Volcano plot analysis for the GSE56815 dataset

Supplemental Fig. 2. Establishment and validation of ZFP36-modified BMSCs. (A) The surface markers of isolated BMSCs by flow cytometry. (B) WB analysis of ZNF36 in hBMSCs with shRNA lentivirus infection. (C) qPCR analysis of ZNF36 in hBMSCs with shRNA lentivirus infection. (D) WB analysis of ZNF36 in hBMSCs with overexpression and shRNA lentivirus infection. (E) qPCR analysis of ZNF36 in hBMSCs with overexpression and shRNA lentivirus infection. Data represent means $\pm$ SD. * $P < 0.05$, and ** $P < 0.01$

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Author contributions

HR.S designed and performed the research; HR.S, XL.Y, and BX.Z analyzed and interpreted the data, and drafted the manuscript; LY.L and JL.W collected the data and designed the methodology; HR.S, XL.Y, and JL.W reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare that there is no conflict interest.

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References

1. Srivastava M, Deal C. Osteoporosis in elderly: prevention and treatment. *Clin Geriatr Med*. 2002;18(3):529–55.
2. LeBoff MS, Greenspan SL, Insogna KL, Lewiecki EM, Saag KG, Singer AJ, Siris ES. The clinician's guide to prevention and treatment of osteoporosis. *Osteoporos Int*. 2022;33(10):2049–102.
3. Ensrud KE, Crandall CJ. Osteoporosis. *Ann Intern Med*. 2017;167(3):ITC17–32.
4. Hu S, Wang S. The role of SIRT3 in the osteoporosis. *Front Endocrinol*. 2022;13:893678.
5. Wang ZX, Luo ZW, Li FX, Cao J, Rao SS, Liu YW, Wang YY, Zhu GQ, Gong JS, Zou JT, et al. Aged bone matrix-derived extracellular vesicles as a messenger for calcification paradox. *Nat Commun*. 2022;13(1):1453.
6. Zhang Z, Guo M, Li Y, Shen M, Kong D, Shao J, Ding H, Tan S, Chen A, Zhang F, et al. RNA-binding protein ZFP36/TTP protects against ferroptosis by regulating autophagy signaling pathway in hepatic stellate cells. *Autophagy*. 2020;16(8):1482–505.
7. Shay JW, Homma N, Zhou R, Naseer MI, Chaudhary AG, Al-Qahtani M, Hirokawa N, Goudarzi M, Fornace AJ, Baesa S, et al. Abstracts from the 3rd international genomic Medicine Conference (3rd IGM 2015): Jeddah, Kingdom of Saudi Arabia. *BMC Genomics*. 2016;17 Suppl 6(Suppl 6):487. 30 November – 3 December 2015.
8. Zhang H, Feng J, Lin Z, Wang S, Wang Y, Dai S, Kong W, Wang Y, Zhang Z. Identification and analysis of genes underlying bone Mineral density by integrating microarray data of osteoporosis. Volume 8. *Front Cell Dev Biol*; 2020. p. 798.
9. Hulse EJ, Haslam JD, Emmett SR, Woolley T. Organophosphorus nerve agent poisoning: managing the poisoned patient. *Brit J Anaesth*. 2019;123(4):457–63.
10. Ye F, Li J, Xu P, Xie Z, Zheng G, Liu W, Ye G, Yu W, Lin J, Su Z, et al. Osteogenic differentiation of mesenchymal stem cells promotes c-Jun-dependent secretion of interleukin 8 and mediates the migration and differentiation of CD4(+) T cells. *Stem Cell Res Ther*. 2022;13(1):58.
11. Fischer V, Haffner-Luntzer M. Interaction between bone and immune cells: implications for postmenopausal osteoporosis. *Semin Cell Dev Biol*. 2022;123:14–21.
12. Yu W, Zhong L, Yao L, Wei Y, Gui T, Li Z, Kim H, Holdreith N, Jiang X, Tong W et al. Bone marrow adipogenic lineage precursors promote osteoclastogenesis in bone remodeling and pathologic bone loss. *J Clin Invest* 2021, 131(2).
13. Wang X, Liang T, Zhu Y, Qiu J, Qiu X, Lian C, Gao B, Peng Y, Liang A, Zhou H, et al. Melatonin prevents bone destruction in mice with retinoic acid-induced osteoporosis. *Mol Med*. 2019;25(1):43.
14. Duarte-Conde JA, Sans-Coll G, Merchante C. RNA-binding proteins and their role in translational regulation in plants. *Essays Biochem*. 2022;66(2):87–97.
15. Shi DL. RBM24 in the Post-transcriptional Regulation of Cancer Progression: Anti-tumor or Pro-tumor Activity? *Cancers* 2022, 14(7).
16. Shukla S, Elson G, Blackshear PJ, Lutz CS, Leibovich SJ. 3'UTR AU-Rich elements (AREs) and the RNA-Binding protein tristetraprolin (TTP) are not required for the LPS-Mediated destabilization of phospholipase-Cbeta-2 mRNA in murine macrophages. *Inflammation*. 2017;40(2):645–56.
17. Wang J, Fu Q, Yang J, Liu JL, Hou SM, Huang X, Cao JS, Liu TL, Wang KZ. RNA N6-methyladenosine demethylase FTO promotes osteoporosis through demethylating Runx2 mRNA and inhibiting osteogenic differentiation. *Aging*. 2021;13(17):21134–41.
18. Wang W, Li T, Feng S. Knockdown of long non-coding RNA HOTAIR promotes bone marrow mesenchymal stem cell differentiation by sponging microRNA miR-378 g that inhibits nicotinamide N-methyltransferase. *Bioengineered*. 2021;12(2):12482–97.
19. Stumpo DJ, Byrd NA, Phillips RS, Ghosh S, Maronpot RR, Castranio T, Meyers EN, Mishina Y, Blackshear PJ. Chorionallantoic fusion defects and embryonic lethality resulting from disruption of Zfp36L1, a gene encoding a CCH zinc finger protein of the tristetraprolin family. *Mol Cell Biol*. 2004;24(14):6445–55.
20. Khalaj K, Luna RL, de Franca ME, de Oliveira WH, Peixoto CA, Tayade C. RNA binding protein, tristetraprolin in a murine model of recurrent pregnancy loss. *Oncotarget*. 2016;7(45):72486–502.
21. Brennan-Laun SE, Li XL, Ezelle HJ, Venkataraman T, Blackshear PJ, Wilson GM, Hassel BA. RNase L attenuates mitogen-stimulated gene expression via transcriptional and post-transcriptional mechanisms to limit the proliferative response. *J Biol Chem*. 2014;289(48):33629–43.
22. Moore MJ, Blachere NE, Fak JJ, Park CY, Sawicka K, Parveen S, Zucker-Scharff I, Moltedo B, Rudensky AY, Darnell RB. ZFP36 RNA-binding proteins restrain T cell activation and anti-viral immunity. *Elife* 2018, 7.
23. Cicchetti AC, Jacobson EC, Sunshine H, Wilde BR, Krall AS, Jarrett KE, Sedgeman L, Turner M, Plath K, Iruela-Arispe ML, et al. ZFP36-mediated mRNA decay regulates metabolism. *Cell Rep*. 2023;42(5):112411.

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