

## Original Research

# Hsa\_circFOXP1 Contributes to Breast Cancer Progression through Regulating miR-338-3p

Kaiping Wu<sup>1</sup>, Kunkun Cheng<sup>2,\*</sup><sup>1</sup>First Clinical Medical College, Nanjing Medical University, 210009 Nanjing, Jiangsu, China<sup>2</sup>Department of Nursing, Jiangsu Cancer Hospital/Jiangsu Institute of Cancer Research/The Affiliated Cancer Hospital of Nanjing Medical University, 210009 Nanjing, Jiangsu, China\*Correspondence: [chengkunkundr@163.com](mailto:chengkunkundr@163.com) (Kunkun Cheng)

Academic Editors: Valerio Gaetano Vellone and Michael H. Dahan

Submitted: 8 December 2023 Revised: 17 January 2024 Accepted: 30 January 2024 Published: 11 May 2024

## Abstract

**Background:** Most patients with advanced-stage breast cancer present with bone metastasis, which seriously affects their quality of life and prognosis. We aimed to investigate the potential role of and the mechanism of circular Forkhead box protein P1 (FOXP1) (hsa\_circ\_0008234) in bone metastasis of breast cancer. **Methods:** The Gene Expression Omnibus database (GEO) database (GSE111504) was used to screen the differentially expressed circular RNAs (circRNAs) in metastatic breast cancer. The related expression of circular FOXP1 (circFOXP1) and miRNA was measured by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). Cellular experiments were performed to assess the influence of circFOXP1 in breast cancer cells. After co-culture of circFOXP1 siRNA-transfected MDA-MB-231 cells and bone marrow-derived mesenchymal stem cells (BMSCs), the effect of circFOXP1 on osteogenic genes was detected. Bioinformatic Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed using clusterProfiler 4.8.2 and R package version 4.3. **Results:** circFOXP1 was upregulated in patients with breast cancer, particularly in bone metastasis breast cancer. Silencing of circFOXP1 decreased the abilities of proliferation, migration, and invasion. The increased alkaline phosphatase (ALP) activity and osteogenic gene expression of BMSCs co-cultured with the MDA-MB-231/si-circRNA group was observed. miR-338-3p was a target miRNA of circFOXP1. Bioinformatic enrichment analysis indicated that the targeted mRNAs were involved in MAPK pathway, regulation of actin cytoskeleton, tight junction, Ras pathway, and PI3K-AKT pathway. **Conclusions:** circFOXP1 upregulation was related to bone metastasis of breast cancer. Silencing of circFOXP1 in breast cancer cells might repress breast cancer cellular activities and facilitate osteogenic differentiation of BMSCs in the microenvironment by targeting miR-338-3p. circFOXP1 might be a therapeutic target for patients with bone metastasis of breast cancer.

**Keywords:** hsa\_circ\_0008234; breast cancer; bone metastasis; miR-338-3p; bioinformatics

## 1. Introduction

The incidence of breast cancer in women is increasing annually and is displaying a tendency to affect younger women [1]. Breast cancer is a malignant tumor prone to distant metastasis, some cases report distant metastasis during the initial diagnosis [2]. Breast cancer metastasis is organ-selective and bone metastasis occurs in approximately 75% of these patients [3]. Bone metastasis of breast cancer can lead to osteolytic bone damage, and often cause a series of bone-related events, such as pathogenic fracture, nerve compression (including spinal cord compression), hypercalcemia, and limb dysfunction, which seriously affects the quality of life of patients and even leads to death [4]. Comprehensive treatment (surgery, radiotherapy, endocrine therapy, chemotherapy, and targeted therapy) is principally used for bone metastasis of breast cancer, and predominantly aims to improve survival [5]. Therefore, it is particularly important to explore the molecular mechanism underlying the bone metastasis of breast cancer and identify effective therapeutic targets.

Several genes or molecules with a regulatory role are expressed aberrantly during malignant tumor development, which may serve as novel targets or biomarkers, and circular RNAs (circRNAs) are one of the research hotspots [6,7]. circRNAs refer to a group of endogenous non-coding RNAs characterized by covalently closed-loop structures [8]. The aberrant expression of circRNAs results in potential diagnostic or prognostic performance in tumors [9,10]. Some circRNAs are potential therapeutic targets for cancers and bone metastatic treatment [11,12]. For instance, circIKBKB is upregulated in patients with bone-metastasis of breast cancer and can facilitate bone metastasis by activating the NF- $\kappa$ B pathway [12]. circular Forkhead box protein P1 (circFOXP1) (also named hsa\_circ\_0008234) is derived from *Forkhead box protein P1 (FOXP1)* and located in chr3:71090478-71102924. circFOXP1 is expressed abnormally and participates in tumorigenesis of several types of cancers, such as lung cancer, cutaneous squamous cell carcinoma, and gallbladder cancer [13–15]. However, the function of circFOXP1 in breast cancer has not yet been determined.



This study detected and screened the circRNAs expressed differentially in metastatic breast cancer using the Gene Expression Omnibus database (GEO) GSE111504. The expression of circFOXPI was validated in breast cancer tissues (BCa/T) and those with bone metastasis (BM-BCa/T). Subsequently, the function of circFOXPI in breast cancer cells was evaluated. Bioinformatic analysis was performed to predict the potential miRNAs of circFOXPI. The predicted target genes were used to conduct the protein-protein interaction (PPI) network, and to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrich analysis.

## 2. Materials and Methods

### 2.1 Clinical Sample Collection

The sample collection was based on the specimen bank of Nanjing Medical University. The study population included patients with breast cancer who underwent surgery at our hospital from May 2019 to January 2023, including 60 patients with bone metastasis of breast cancer and 110 patients with primary breast cancer. The samples of bone metastasis, primary lesions, and adjacent breast cancer tissues were obtained during the surgery. All the samples were quickly frozen in liquid nitrogen. All the patients and their families were informed of the study content and signed an informed written consent form. The study was approved by the Ethics Committee of the Jiangsu Cancer Hospital (Approval No. 2019017).

### 2.2 Bioinformatic Analysis

The circRNAs expressed differentially in metastatic breast cancer were screened from the GEO database GSE111504. GSE111504 circRNA expression profile was based on GPL21825 platform from parental MDA-MB-231 (231-PAR) cells, isogenic brain metastatic cells, and metastatic cells. The volcano map was conducted based on the condition of  $|\log_{2}FC| \geq 1$  and  $p < 0.05$ . The online circInteractome [16] and circBank [17] databases were used to predict the targeting miRNAs of circFOXPI and overlapped them using jvenn [18]. The binding miRNAs of miR-338-3p were derived from Targetscan [19], miRDB [20], miRWalk (<http://mirwalk.ummm.uni-heidelberg.de/>), and starBase [21]. String and Cytoscape were used to analyze the PPI network and hub genes. The GO and KEGG enrichment analysis (<https://www.bioinformatics.com.cn/>) were performed using clusterProfiler 4.8.2 and R package version 4.3 [22,23].

### 2.3 Cell Culture, Treatment, and Transfection

Human breast cancer cells MCF-7 (HTB-22), SK-BR-3 (HTB-30), T47D (HTB-133), and MDA-MB-231 (CRM-HTB-26), as well as non-tumorigenic epithelial cell line MCF-10A (CRL-10317), were acquired from American Type Culture Collection (ATCC; Manassas, VA, USA) with short tandem repeats (STR) verification. All can-

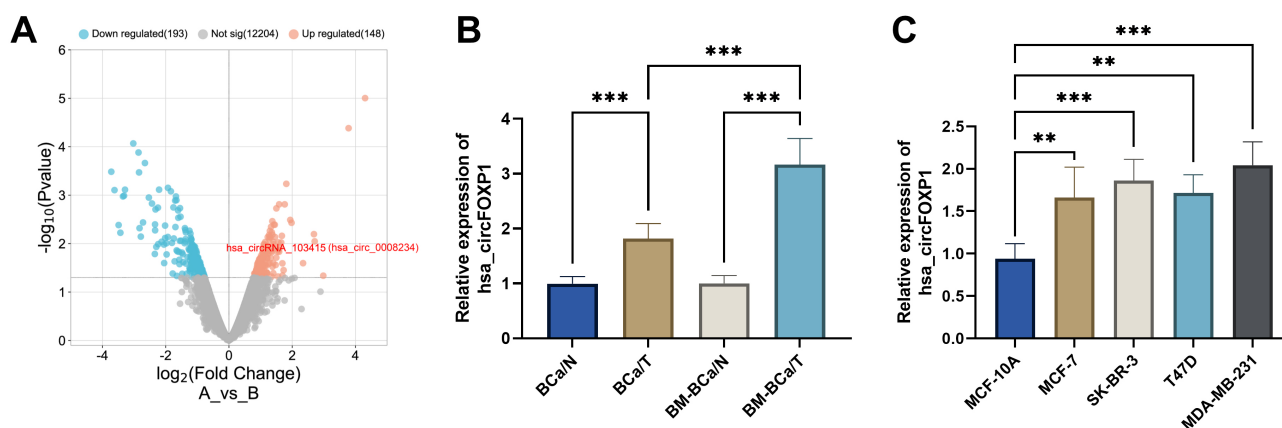
cer cell lines were maintained in liquid nitrogen and incubated in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) at 37 °C in a 5% CO<sub>2</sub> humidified incubator. MCF-10A cells were maintained in DMEM/F12 medium with 5% equine serum (heat-inactivated). Bone marrow mesenchymal stem cells (BMSCs) were purchased from Cyagen Biotechnology (Santa Clara, CA, USA) and maintained in a low-glucose DMEM medium with 10% FBS. The mycoplasma detection was carried out on a regular basis every one to three months using MycAway™ Plus-Color One-Step Mycoplasma Detection Kit (Yeasen Biotechnology, Shanghai, China).

circFOXPI siRNAs (si-circRNA-1, -2, -3), siRNA negative control (si-NC), miR-338-3p mimic (miR-338-3p), and mimic NC (miR-NC) were synthesized by GenePharma (Shanghai, China). Transfection of plasmids into breast cancer cells was performed with the help of lipofectamine 2000 (Invitrogen, Grand Island, NY, USA). After 48 h of transfection, a real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed to detect the transfection efficiency. The sequences for circFOXPI siRNAs were as follows: si-circRNA-1, 5'-AGGGAAAGGTTCCCGTGTCTAG-3'; si-circRNA-2, 5'-CCAAAAGGGAAAGGTTCCCGT-3'; si-circRNA-3, 5'-GAAAGGTTCCCGTGTCTAGTGG-3'; for si-NC, 5'-UUCUCCGAACGUGUCACGUTT-3'; miR-338-3p mimic, 5'-UCCAGCAUCAGUGAUUUUGUUG-3'; and mimic NC, 5'-CAGUAC UUUUAGUGUGUACAA-3'.

The co-culture model was performed by applying the medium supernatant of different transfected cells to the BMSCs culture. The co-cultured BMSCs were incubated for 1, 3, 5, 7, and 9 days to detect alkaline phosphatase (ALP) activity.

### 2.4 RNA Extraction, Reverse Transcription, and RT-qPCR Experiments

The clinical samples were cut into sizes measuring approximately 0.5 × 0.5 × 0.5 cm and were placed in 1.5 mL Eppendorf tubes. The supernatant of the cultured cells was discarded and rinsed thoroughly with phosphate buffered saline (PBS). Then Trizol reagent (Takara, Dalian, Liaoning, China) was added to the tissue samples and cells to extract total RNAs. Total RNA (treated with RNase R for circRNA/without RNase R for mRNA) was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (ThermoFisher, Applied Biosystems, Waltham, MA, USA). qPCR was performed using SYBR Green PCR Master Mix (Vazyme, Nanjing, Jiangsu, China) on a 7900HT Fast Real-Time PCR system. Finally, GAPDH was used as a reference to calculate the relative content of the target circFOXPI and genes. The PCR sequences for circFOXPI were forward 5'-CCTCCTCTGCACCTTCCAAG-3' and reverse 5'-TGTTGCTGGAGGATCTGCTG-3'; for GAPDH were:



**Fig. 1. circFOX P1 expression in breast cancer.** (A) Volcano plot of circRNAs from GES111504 and screened upregulated circFOX P1 (hsa\_circ\_0008234). (B) circFOX P1 expression was detected in breast cancer bone metastasis tissues, primary breast cancer tissues, and surrounding tissues. (C) circFOX P1 expression was increased in breast cancer cells in contrast with that in normal MCF-10A cells.  $**p < 0.01$ ,  $***p < 0.001$ . FOX P1, Forkhead box protein P1; circRNAs, circular RNAs; BCa/T, breast cancer tissues; BM-BCa/T, bone metastasis breast cancer tissues; BCa/N, breast cancer adjacent normal tissues; BM-BCa/N, bone metastatic breast cancer adjacent normal tissues.

forward 5'-GAGTCAACGGATTTGGTCGT-3' and reverse 5'-TTGATTTTGGAGGCATCTCG-3'.

## 2.5 Western Blot (WB) Assay

After co-culture, the cells were collected and homogenized with RIPA lysis buffer to obtain total protein. The protein purity and concentration were measured by standard methods of BCA assays (Beyotime, Shanghai, China). 10  $\mu\text{g}$  per sample were separated by SDS-PAGE for 90 min, then blotted onto nitrocellulose membranes for 1 h, and incubated with primary antibodies (Abcam, Cambridge, UK) overnight at 4  $^{\circ}\text{C}$ . Nitrocellulose membranes were washed four times and treated with appropriate secondary HRP-conjugated goat anti-rabbit or goat anti-mouse antibodies (Abcam, Cambridge, UK) at 37  $^{\circ}\text{C}$  for 1 h. Finally, membranes were scanned and transferred to the Image J program (version 1.53, National Institutes of Health, Bethesda, MD, USA) for gel gray measurement.

## 2.6 Cell Proliferation Assay and ALP Detection

The cell proliferative capacities were assessed using cell counting kit-8 (CCK-8, Dojindo, Tokyo, Japan). The transfection-treated breast cancer cells were incubated in 96-well plates (approximately 5000 cells/well). The CCK-8 reagent was added to each well at 0, 24, 48, and 72 h time points. The cells were incubated for further 1 h. The microplate Reader (Bio-Rad, Hercules, CA, USA) was used to determine the absorbance at a wavelength of 450 nm (OD value).

ALP activity of co-cultured BMSCs was measured using an Alkaline Phosphatase Activity Assay Kit (Beyotime, Shanghai, China).

## 2.7 Transwell Assays

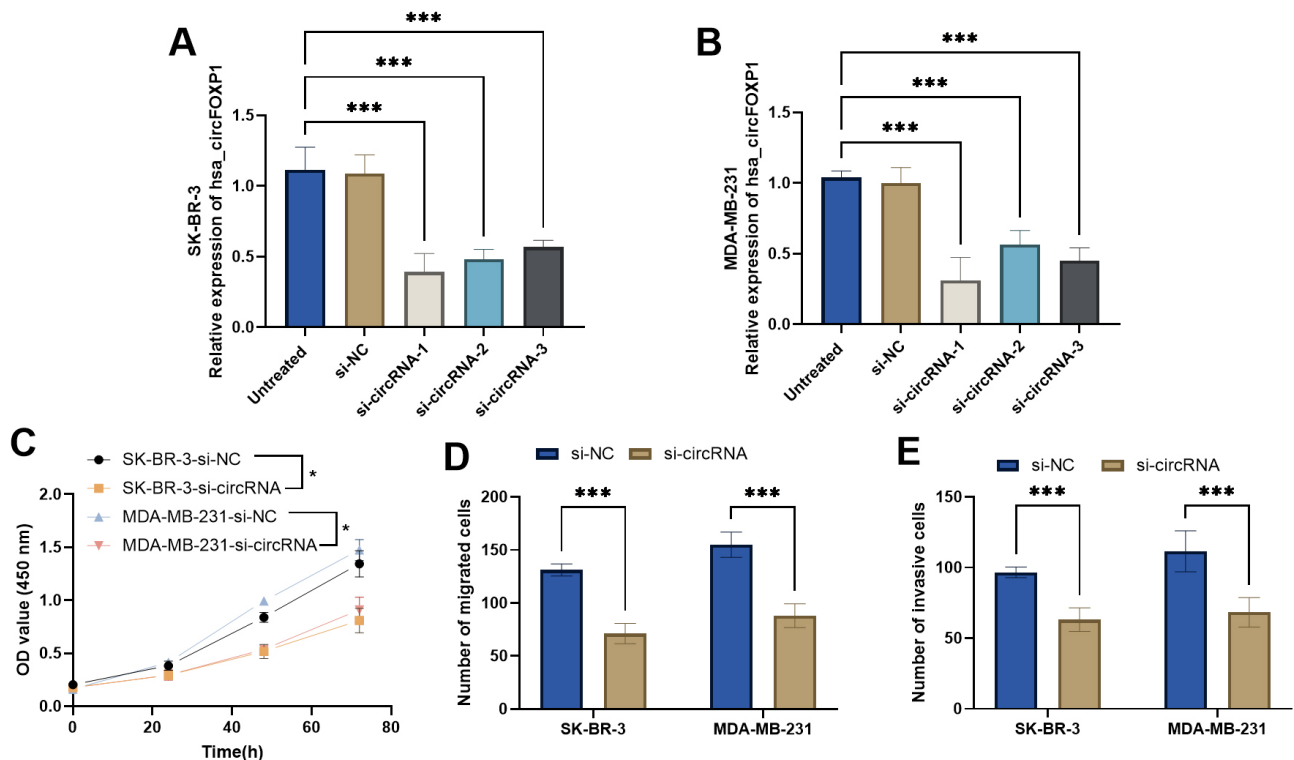
DMEM medium without serum was put on the upper layer of the Transwell culture plate (pore size = 8  $\mu\text{m}$ ) coated with or without Matrigel (Corning, Corning, NY, USA), and DMEM medium containing 10% FBS was added to the lower layer. The cell suspension ( $5 \times 10^4$  cells/well) was added to Transwell plates and incubated for 24 h at 37  $^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  incubator. The filter membrane was removed, and the cells that migrated or invaded the lower layer were stained and counted under a microscope.

## 2.8 Dual-luciferase Report Assay

The circFOX P1-wild type (WT-circRNA) or circFOX P1-mutant (MUT-circRNA) binding sites with miR-338-3p were inserted into the pGL3 promoter vector (Realgene, Shanghai, China). Breast cancer cells were cultured and seeded in 24-well plates. miR-338-3p mimic, mimic NCs, and luciferase reporter plasmids (WT-circRNA or MUT-circRNA) were co-transfected into the cells when cells grew to 80% confluence. After 48 h of incubation, firefly luciferase activities were measured using Dual-luciferase Reporter Assay System (Promega, Madison, WI, USA).

## 2.9 Statistical Analysis

The statistical data are expressed as mean  $\pm$  standard deviation (SD) and analyzed using GraphPad 9.0 software (Dotmatics, Boston, MA, USA). All experiments repeated at least three times. One-way analysis of variance (ANOVA) or two-way ANOVA was performed for comparison among groups and the Student *t*-test was used for pairwise comparison.  $p < 0.05$  indicates statistical significance.



**Fig. 2. Functional role of circFOX P1 in breast cancer cells.** (A,B) circFOX P1 siRNAs decrease circFOX P1 expression in SK-BR-3 and MDA-MB-231 cells. (C) CCK-8 assay measured the influence of circFOX P1 siRNA in breast cancer cells. (D,E) circFOX P1 interference decreased breast cancer cell migration (D) and invasion (E) abilities. \* $p < 0.05$ , \*\*\* $p < 0.001$ . CCK-8, cell counting kit-8; si-NC, siRNA negative control; si-circRNA, circFOX P1 siRNA.

### 3. Results

#### 3.1 Identification and Validation of circFOX P1 Expression in Metastatic Breast Cancer

According to GSE111504 circRNA profile data, the volcano map was based on the condition of  $|\log FC| \geq 1$  and  $p < 0.05$ . circFOX P1 (hsa\_circ\_0008234) was one of the upregulated circRNAs in metastatic breast cancer cells (Fig. 1A). We measured the circFOX P1 expression in breast cancer tissues using RT-qPCR. circFOX P1 expression was high in breast cancer tissues (BCa/T), particularly in tissues with bone metastasis of breast cancer (BM-BCa/T) compared with adjacent normal tissues (BCa/N and BM-BCa/N) ( $p < 0.001$ , Fig. 1B). Furthermore, circFOX P1 levels were higher in breast cancer cells, compared with normal MCF-10A cells ( $p < 0.01$ , Fig. 1C).

#### 3.2 Functional Implication of circFOX P1 in Breast Cancer Cells

circFOX P1 siRNAs (si-circRNA-1, si-circRNA-2, si-circRNA-3) were transfected into SK-BR-3 MDA-MB-231 cells. Fig. 2A,B depict that si-circRNA-1 exhibited the highest inhibitory effect on circFOX P1 expression ( $p < 0.001$ ). Thus, si-circRNA-1 (termed si-circRNA) was used for subsequent experiments. CCK-8 assay results suggested that interference of circFOX P1 decreased the cell prolifera-

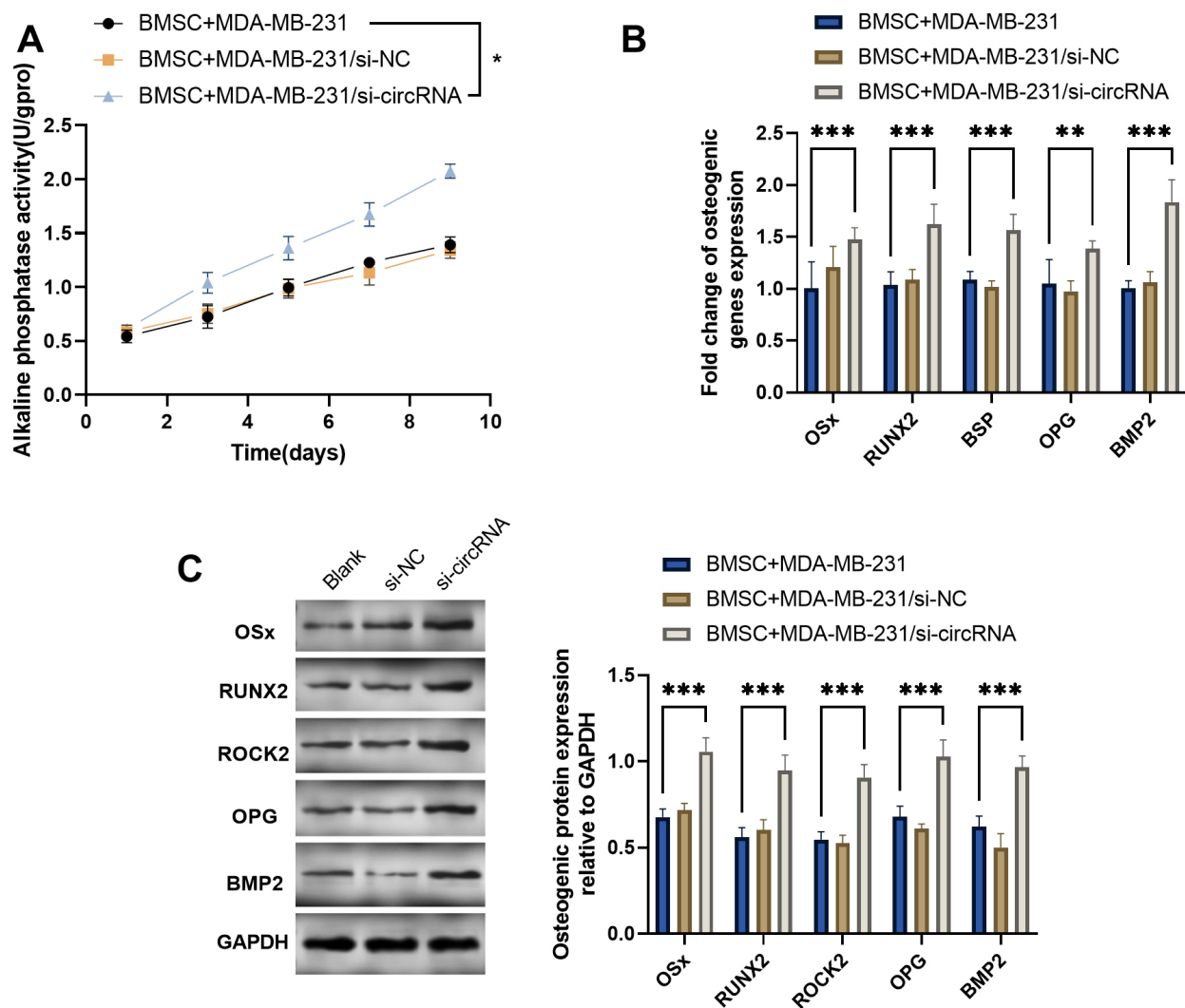
tive abilities ( $p < 0.05$ , Fig. 2C). A similar inhibitory trend caused by si-circFOX P1 was observed in migration assay ( $p < 0.001$ , Fig. 2D) and invasion assay ( $p < 0.001$ , Fig. 2E).

#### 3.3 Relationship between circFOX P1 and the Osteogenic Differentiation of BMSCs in Cell Microenvironment

To investigate the effects of circFOX P1 on the interaction between breast cancer cells and BMSCs, the supernatant of MDA-MB-231 cells cultured in si-circRNA group and si-NC group were collected and co-cultured with BMSCs. The increased ALP activity of BMSCs co-cultured with MDA-MB-231/si-circRNA group was observed ( $p < 0.05$ , Fig. 3A). Fig. 3B,C depicted that the five osteogenic genes/proteins of *OSX*, *RUNX2*, *BSP*, *OPG*, and *BMP2* expression were increased in BMSC + MDA-MB-231/si-circRNA group contrast with control group ( $p < 0.01$ ).

#### 3.4 miR-338-3p as a Target miRNA of circFOX P1

Two circRNA-miRNA interaction databases (circInteractome and circBank) were used to screen the intersection of miRNAs that may play regulatory roles. These databases overlapped five potential miRNAs (Fig. 4A). Among the five miRNAs, miR-338-3p exhibited the highest levels after silencing of circFOX P1 in MDA-MB-231 cells, which was selected to validate the targeting relationship with circFOX P1 ( $p < 0.001$ , Fig. 4B). Fig. 4C displays the



**Fig. 3. circFOXPI silencing in MDA-MB-231 improves the BMSCs osteogenesis.** (A) Alkaline phosphatase activity analysis of BMSCs co-cultured with transfected MDA-MB-231 culture supernatant. (B) The osteogenic genes *OSx*, *RUNX2*, *BSP*, *OPG*, and *BMP2* in BMSCs after co-culture. (C) Osteogenic proteins expression of BMSCs after co-culture. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . BMSCs, bone marrow-derived mesenchymal stem cells.

binding sites between circFOXPI and miR-338-3p. Dual-luciferase reporter assay indicated that miR-338-3p overexpression suppressed the luciferase activities of circFOXPI wild-type of MDA-MB-231 cells ( $p < 0.001$ , Fig. 4D).

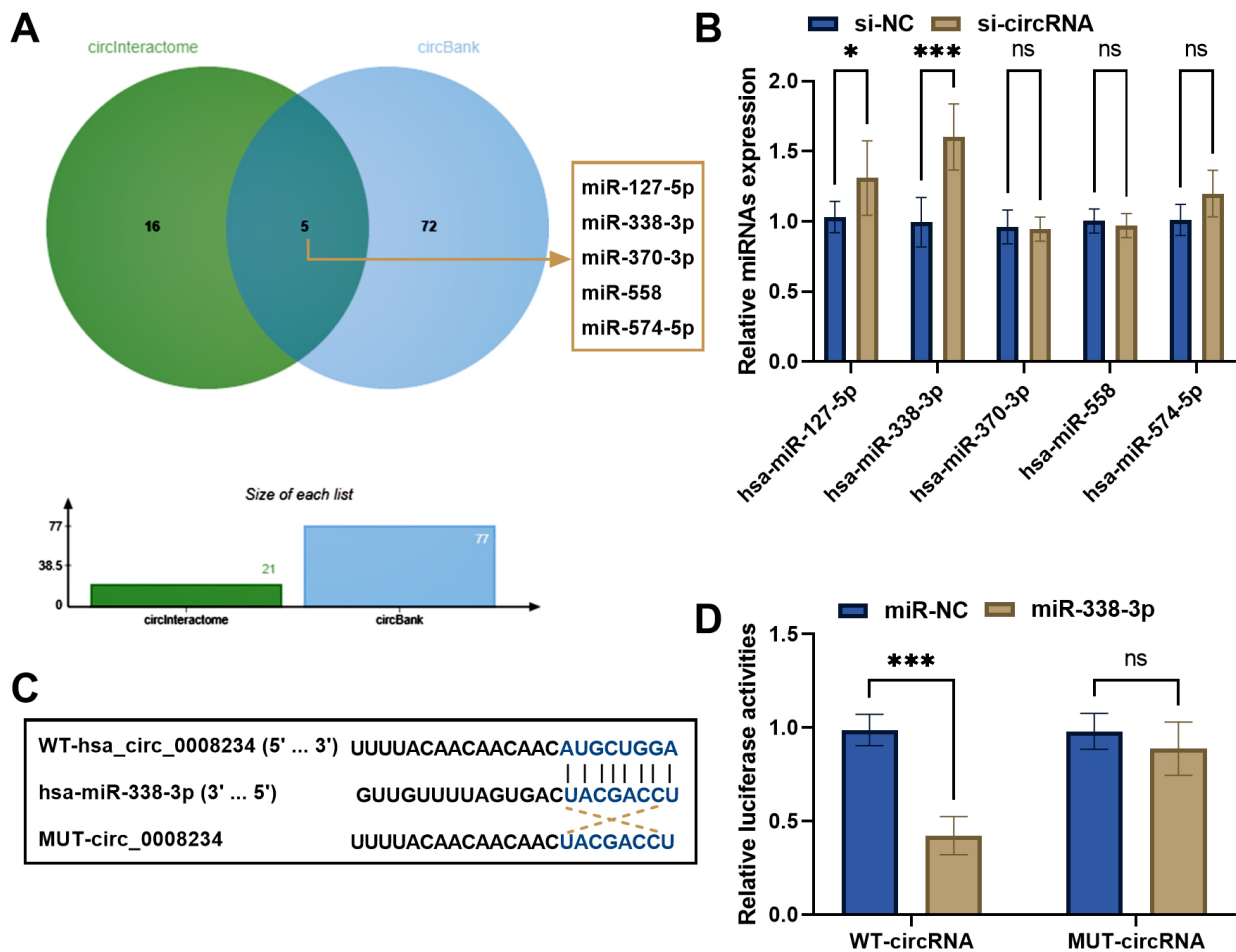
### 3.5 Potential Targets of miR-338-3p and Enrichment Analysis

To explore the potential molecular mechanism underlying circFOXPI, TargetScan, miRDB, miRWalk, and starBase algorithms were used to overlap the potential target mRNAs of miR-338-3p (Fig. 5A). The STRING online database predicted PPI using the overlapped 92 mRNAs of miR-338-3p (Fig. 5B) and the top ten target mRNAs (PTEN was the leading mRNA) were visualized by Cytoscape (Fig. 5C). The GO (Fig. 5D) and KEGG (Fig. 5E) pathway enrichment analysis indicated that the targets genes were

principally enriched in dendrite development, morphogenesis, and other biological processes, and pathway enriched in MAPK signaling pathway, regulation of actin cytoskeleton, tight junction, Ras signaling pathway, and PI3K-AKT signaling pathway.

## 4. Discussion

Most patients with breast cancer develop bone metastasis during the disease course, and the treatment regimens are unsatisfactory, so it is crucial to seek novel therapeutic targets for treating breast cancer, particularly for patients with bone metastasis of breast cancer. The GSE111504 dataset exhibited upregulated circFOXPI in metastatic breast cancer cells. Aberrant expression of circFOXPI participated in the tumorigenesis of several types of cancers [13,14]. However, the detailed function of cir-



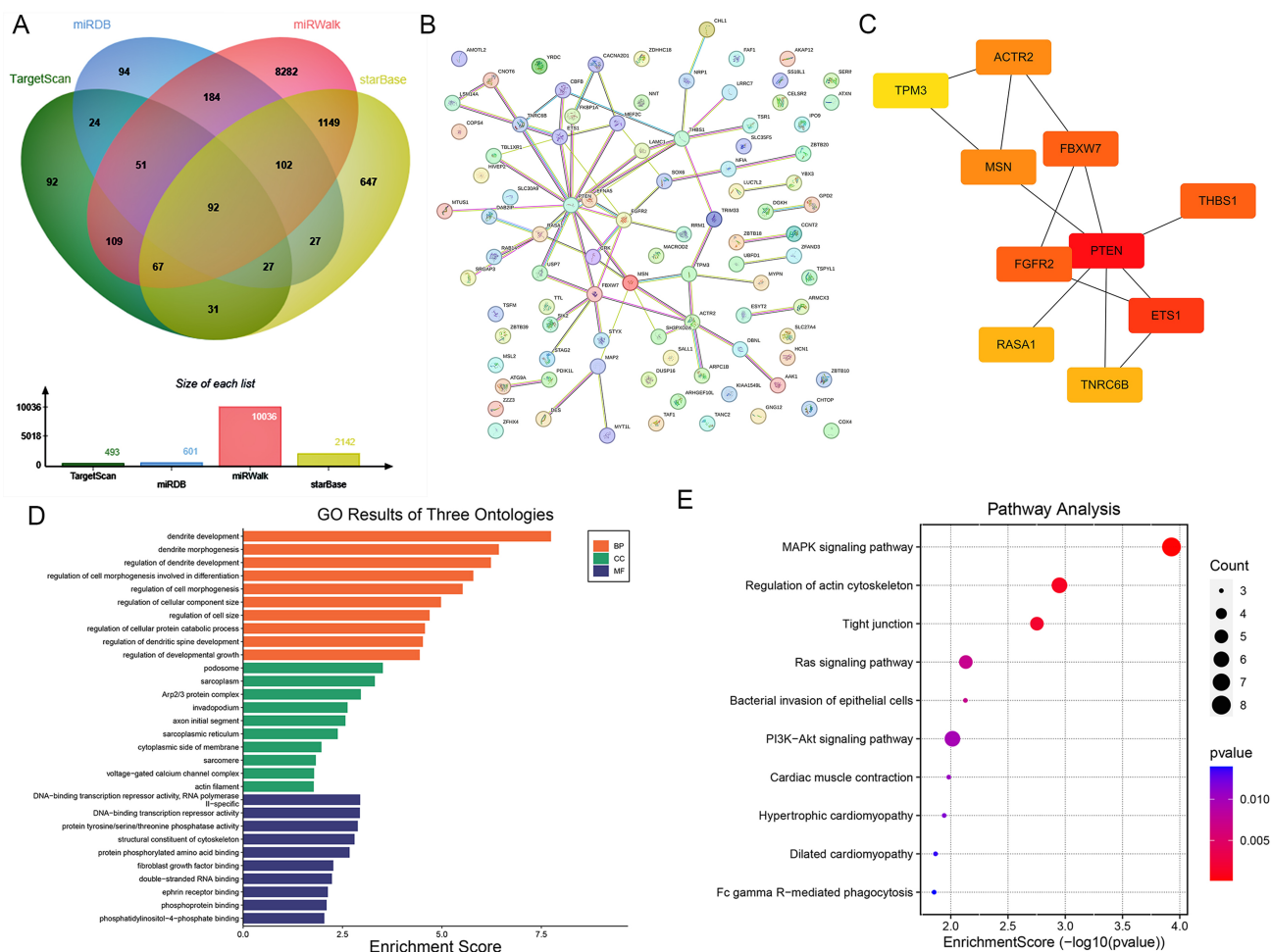
**Fig. 4. circFOX P1 could sponge miR-338-3p.** (A) circInteractome and circBank databases overlapped the potential predictive miRNAs of circFOX P1. (B) Relative expression of miRNAs was measured in si-circFOX P1 transfected MDA-MB-231 cells. (C) Binding sites between circFOX P1 and miR-338-3p. (D) Dual-luciferase reporter assay confirmed the relationship between circFOX P1 and miR-338-3p. \* $p < 0.05$ , \*\*\* $p < 0.001$ , ns, no significance. WT-circRNA, circFOX P1-wild type; MUT-circRNA, circFOX P1-mutant; miR-NC, mimic NC; miR-338-3p, miR-338-3p mimic.

cFOX P1 in breast cancer, particularly in bone metastasis remains elusive. Here, the circFOX P1 levels were increased in metastatic breast cancer tissues, especially in bone metastatic breast cancer tissues, as well as breast cancer cells. Interfering circFOX P1 expression could repress cellular activities, including proliferative capacities, migration abilities, and invasion potential. Importantly, silencing of circFOX P1 could increase ALP activities and osteogenic gene expression. Bioinformatic analysis displayed that the network genes were enriched in several signaling pathways related to bone metastasis.

circFOX P1 was overexpressed in breast cancer tissues and cells, particularly in bone metastatic breast cancer tissues, suggesting the potentially crucial role of circFOX P1 in the tumorigenesis of breast cancer and bone homeostasis. The functional significance of circFOX P1 has been reported in several types of cancers, including the promoting role of circFOX P1 in cutaneous squamous carcinoma by facilitating cell proliferation through miR-127-

5p/*ADCY7* axis regulation [13]. circFOX P1 has identified as a prognostic biomarker and regulator in gallbladder cancer progression and the Warburg effect by regulating miR-370/*PKLR* expression [15]. Besides, the inconsistent role of circFOX P1 in different subtypes of lung cancer was reported, which may be attributed to the circFOX P1 expression measurement in different samples [14,24,25]. In this study, the functional performance of circFOX P1 was explored. Silencing of circFOX P1 displayed anti-neoplastic properties through inhibiting proliferative, migratory, and invasive potentials, suggesting that circFOX P1 might play a tumor-promoting role in breast cancer tumorigenesis.

The concentration of bone metabolic markers for numerous bone disease diagnoses, the assessment of fracture risk prediction, and drug efficacy, are valuable. The present study tried to measure the ALP activities and osteogenic genes (*OSX*, *RUNX2*, *BSP*, *OPG*, and *BMP2*) expression in BMSCs co-cultured with MDA-MB-231/si-circRNA group. *OSX*, *RUNX2*, *BSP*, and *BMP2* could re-



**Fig. 5. Downstream mRNAs of miR-338-3p and pathway enrichment analysis.** (A) Downstream mRNAs of miR-338-3p were overlapped using four online databases. (B) STRING conducted a protein-protein interaction (PPI) network using overlapped 92 mRNAs. (C) The leading ten hub genes were identified using the Cytoscape network. (D) Gene Ontology (GO) analysis was performed using the related genes in the network. (E) Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway enrichment analysis of the genes in the network genes.

flect osteoblastic differentiation ability and *OPG* was identified as a refection marker of osteoclastogenesis [26,27]. Increased ALP activities and osteogenic gene expression in BMSCs co-cultured with MDA-MB-231/si-circRNA group illustrated that circFOXP1 silencing in breast cancer might facilitate the osteogenic capacity of BMSCs in the microenvironment of bone metastasis of breast cancer.

The dysregulation of circRNAs may be a key factor in biological processes such as post-transcriptional regulation and epigenetic modification of malignant tumors by regulating miRNA expression. The current study observed that miR-338-3p was a target of circFOXP1, illustrating that circFOXP1 may participate in tumorigenesis of breast cancer by targeting miR-338-3p. miR-338-3p was a tumor suppressor in breast cancer and could be sponged by circTFF1 [28,29]. In addition, osteogenic gene *RUNX2* might be a target of miR-338-3p, other osteogenic genes did not show targeting relationship with miR-338-3p (Supplementary

**Fig. 1).** These data revealed that circFOXP1/miR-338-3p might affect the osteogenic genes expression by modulating breast cancer bone metastasis, but it might not directly regulate the expression of osteogenic genes through miR-338-3p. Furthermore, the potential mechanism underlying circFOXP1 was explored using bioinformatic enrichment analysis. Among the overlapped targeted mRNAs of miR-338-3p, *PTEN* was the top hub gene. In addition, the targeted genes were related to biological processes (BP) of dendrite development, podosome cellular processes (CC), and DNA-binding transcription repressor activity, RNA polymerase II-specific molecular function (MF). Plasmacytoid dendritic cells were increased with bone metastasis in breast cancer [30]. Pathway enrichment analysis displayed that the targeted mRNAs were involved in various functional pathways, such as MAPK pathway, regulation of actin cytoskeleton, tight junction, Ras pathway, and PI3K-AKT pathway. These pathways were closely related to bone

metastasis in tumors [31–33]. As a complex pathological process, multiple signaling may control the distant metastasis of breast cancer, and the detailed mechanism underlying circFOXP1 in bone metastasis of breast cancer warrants to further investigation.

## 5. Conclusions

In conclusion, circFOXP1 upregulation was related to bone metastasis of breast cancer. Silencing of circFOXP1 in breast cancer cells can repress breast cancer cellular activities and facilitate osteogenetic differentiation of BMSCs in the microenvironment by targeting miR-338-3p. circFOXP1 might be a therapeutic target for patients with bone metastasis of breast cancer.

## Abbreviations

ALP, alkaline phosphatase; BCa/T, breast cancer tissues; BM-BCa/T, bone metastasis breast cancer tissues; BMSCs, bone marrow mesenchymal stem cells; BP, biological processes; CC, cellular component; circRNAs, circular RNAs; GEO, Gene Expression Omnibus database; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function; miR-NC, mimic NC; RT-qPCR, real-time quantitative reverse transcription polymerase chain reaction; si-NC, siRNA negative control; 231-PAR, parental MDA-MB-231.

## Availability of Data and Materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

## Author Contributions

Both authors designed this study. KW and KC conducted the experiment and analyzed the data. KW wrote the manuscript. KC revised the manuscript. Both authors reviewed and approved for publication.

## Ethics Approval and Consent to Participate

All the enrolled patients and their families were informed of the study content and signed informed written consent. The study was approved by the Ethics Committee of the Jiangsu Cancer Hospital (Approval No. 2019017).

## Acknowledgment

Not applicable.

## Funding

This research received no external funding.

## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.ceog5105112>.

## References

- [1] Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA: a Cancer Journal for Clinicians*. 2023; 73: 17–48.
- [2] Zhang Y, Liang Y, Wang Y, Ye F, Kong X, Yang Q. A novel ferroptosis related gene signature for overall survival prediction and immune infiltration in patients with breast cancer. *International Journal of Oncology*. 2022; 61: 148.
- [3] Tahara RK, Brewer TM, Theriault RL, Ueno NT. Bone Metastasis of Breast Cancer. *Advances in Experimental Medicine and Biology*. 2019; 1152: 105–129.
- [4] Liu C, Wang L, Liu L, Zhuang J, Tang S, Zhang T, *et al*. Efficacy and safety of de-escalation bone-modifying agents for cancer patients with bone metastases: a systematic review and meta-analysis. *Cancer Management and Research*. 2018; 10: 3809–3823.
- [5] Pang L, Gan C, Xu J, Jia Y, Chai J, Huang R, *et al*. Bone Metastasis of Breast Cancer: Molecular Mechanisms and Therapeutic Strategies. *Cancers*. 2022; 14: 5727.
- [6] Puppo M, Taipaleenmäki H, Hesse E, Clézardin P. Non-coding RNAs in bone remodelling and bone metastasis: Mechanisms of action and translational relevance. *British Journal of Pharmacology*. 2021; 178: 1936–1954.
- [7] Chen J, Gu J, Tang M, Liao Z, Tang R, Zhou L, *et al*. Regulation of cancer progression by circRNA and functional proteins. *Journal of Cellular Physiology*. 2022; 237: 373–388.
- [8] Chen L, Wang C, Sun H, Wang J, Liang Y, Wang Y, *et al*. The bioinformatics toolbox for circRNA discovery and analysis. *Briefings in Bioinformatics*. 2021; 22: 1706–1728.
- [9] Chen L, Shan G. CircRNA in cancer: Fundamental mechanism and clinical potential. *Cancer Letters*. 2021; 505: 49–57.
- [10] Zhang M, Bai X, Zeng X, Liu J, Liu F, Zhang Z. circRNA-miRNA-mRNA in breast cancer. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 2021; 523: 120–130.
- [11] Sanjeev G, Pranavkrishna S, Akshaya RL, Rohini M, Selvamurugan N. Regulation of bone metastasis and metastasis suppressors by non-coding RNAs in breast cancer. *Biochimie*. 2021; 187: 14–24.
- [12] Xu Y, Zhang S, Liao X, Li M, Chen S, Li X, *et al*. Circular RNA circIKBKB promotes breast cancer bone metastasis through sustaining NF-κB/bone remodeling factors signaling. *Molecular Cancer*. 2021; 20: 98.
- [13] Cai L, Wang Y, Wu J, Wu G. Hsa\_circ\_0008234 facilitates proliferation of cutaneous squamous cell carcinoma through targeting miR-127-5p to regulate ADCY7. *Archives of Dermatological Research*. 2022; 314: 541–551.
- [14] Luo Y, Zhang Q, Lv B, Shang Y, Li J, Yang L, *et al*. CircFOXP1: A novel serum diagnostic biomarker for non-small cell lung cancer. *The International Journal of Biological Markers*. 2022; 37: 58–65.
- [15] Wang S, Zhang Y, Cai Q, Ma M, Jin LY, Weng M, *et al*. Circular RNA FOXP1 promotes tumor progression and Warburg effect in gallbladder cancer by regulating PKLR expression. *Molecular Cancer*. 2019; 18: 145.
- [16] Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K, Gorospe M. CircInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs. *RNA Biology*. 2016; 13: 34–42.
- [17] Liu M, Wang Q, Shen J, Yang BB, Ding X. Circbank: a comprehensive database for circRNA with standard nomenclature.

RNA Biology. 2019; 16: 899–905.

- [18] Bardou P, Mariette J, Escudié F, Djemiel C, Klopp C. jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics*. 2014; 15: 293.
- [19] McGeary SE, Lin KS, Shi CY, Pham TM, Bisaria N, Kelley GM, *et al*. The biochemical basis of microRNA targeting efficacy. *Science* (New York, N.Y.). 2019; 366: eaav1741.
- [20] Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Research*. 2020; 48: D127–D131.
- [21] Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Research*. 2014; 42: D92–D97.
- [22] Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics: a Journal of Integrative Biology*. 2012; 16: 284–287.
- [23] Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics* (Oxford, England). 2013; 29: 1830–1831.
- [24] Jiang W, He Y, Ma Z, Zhang Y, Zhang C, Zheng N, *et al*. hsa\_circ\_0008234 inhibits the progression of lung adenocarcinoma by sponging miR-574-5p. *Cell Death Discovery*. 2021; 7: 123.
- [25] Ma Y, Zou H. Identification of the circRNA-miRNA-mRNA Prognostic Regulatory Network in Lung Adenocarcinoma. *Genes*. 2022; 13: 885.
- [26] Farshdousti Haghighi M, Noruzinia M, Mortazavi Y, Soleimani M, Kaviani S, Abroun S, *et al*. Different Methylation Patterns of RUNX2, OSX, DLX5 and BSP in Osteoblastic Differentiation of Mesenchymal Stem Cells. *Cell Journal*. 2015; 17: 71–82.
- [27] Zainal Ariffin SH, Lim KW, Megat Abdul Wahab R, Zainal Ariffin Z, Rus Din RD, Shahidan MA, *et al*. Gene expression profiles for *in vitro* human stem cell differentiation into osteoblasts and osteoclasts: a systematic review. *PeerJ*. 2022; 10: e14174.
- [28] Vivacqua A, Sebastiani A, Miglietta AM, Rigracciolo DC, Cirillo F, Galli GR, *et al*. miR-338-3p is regulated by estrogens through GPER in breast cancer cells and cancer-associated fibroblasts (CAFs). *Cells*. 2018; 7: 203.
- [29] Wan L, Han Q, Zhu B, Kong Z, Feng E. Circ-TFF1 Facilitates Breast Cancer Development via Regulation of miR-338-3p/FGFR1 Axis. *Biochemical Genetics*. 2022; 60: 315–335.
- [30] Zhang W, Bado I, Wang H, Lo HC, Zhang XHF. Bone Metastasis: Find Your Niche and Fit in. *Trends in Cancer*. 2019; 5: 95–110.
- [31] Li P, Lin Z, Liu Q, Chen S, Gao X, Guo W, *et al*. Enhancer RNA SLIT2 Inhibits Bone Metastasis of Breast Cancer Through Regulating P38 MAPK/c-Fos Signaling Pathway. *Frontiers in Oncology*. 2021; 11: 743840.
- [32] Song X, Wei C, Li X. The Signaling Pathways Associated With Breast Cancer Bone Metastasis. *Frontiers in Oncology*. 2022; 12: 855609.
- [33] Wang K, Chu Y, Zhang H, Qu X, Wang B, Han Y. FOXD3 suppresses the Proliferation of CRC Bone Metastatic Cells via the Ras/Raf/MEK/ERK Signaling Pathway. *Combinatorial Chemistry & High Throughput Screening*. 2024; 27: 436–445.