

Platelet-rich miR-21 is a predictive biomarker in osteosarcoma and promotes osteosarcoma cell malignancy

Xi'an Pan^{1,2,*}, Shishi Dong^{1,*}, Farui Sun^{1,*}, Yuanjin Zhang¹, Guofu Zhang¹, Jun Li¹ and Bingxia Liu^{3,4}

¹ Department of Orthopaedics, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Huangshi, Hubei, P.R. China

² Hubei Key Laboratory of Kidney Disease Pathogenesis and Intervention, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Huangshi, Hubei, P.R. China

³ Department of Ultrasound imaging, Huangshi Central Hospital Affiliated Hospital of Hubei Polytechnic University, Huangshi, Hubei, P.R. China

⁴ Hubei Key Laboratory of Kidney Disease Pathogenesis and Intervention, Hubei, P.R. China

Abstract. Platelet-derived microRNAs (miRNAs) are regarded as diagnostic biomarkers as well as potent regulators of cancer development. The expression of miR-21, a vital miRNA, is elevated in the tumor tissues of osteosarcoma (OS) patients. However, platelet-derived miR-21 in OS is unknown. Here, we found that the level of miR-21 is increased in the serum of OS patients. The miR-21 level is associated with advanced tumor stages and poorer clinical outcomes in OS patients. Moreover, platelet-derived microvesicles (PMVs) from OS patients (OS-PMVs) significantly enhanced the miR-21 profile in MG63 OS cells. Functional assays revealed that the proliferation and migration of MG63 cells were promoted by OS-PMV incubation or the transfection of miR-21 mimics, whereas the sensitivity of MG63 cells to methotrexate (MTX), doxorubicin (ADM), and cisplatin (DDP) was reduced. However, inhibiting miR-21 partially reversed the OS-PMV-mediated effects. Furthermore, programmed cell death 4 (PDCD4) was identified as a potential target of miR-21 in OS. miR-21 considerably inhibited PDCD4 expression in MG63 cells and reversed the antitumor effects mediated by PDCD4 overexpression. In conclusion, our results suggest that platelet-rich miR-21 may promote the development of OS by targeting PDCD4.

Key words: Osteosarcoma — miR-21 — Platelets — Malignancy — Biomarker

Abbreviations: ADM, doxorubicin; CCK8, Cell Counting Kit-8; DDP, cisplatin; DE, differentially expressed; eIF4A, eukaryotic initiation factor-4A; EMT, epithelial-mesenchymal transition; GEO, Gene Expression Omnibus; GSEA, Gene set enrichment analysis; H-PMVs, PMVs from healthy donors; HSPs, heat shock proteins; LPS, lysophosphatidic acid; miRNAs, microRNAs; MSCs, mesenchymal

* These authors contributed equally to this work.

Electronic supplementary material. The online version of this article (doi: 10.4149/gpb_2025037) contains Supplementary material.

Correspondence to: Jun Li, Department of Orthopaedics, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, No. 141, Tianjin Road, Huangshigang District, Huangshi, Hubei, P. R. China

E-mail: LJ852022@163.com

Bingxia Liu, Department of Ultrasound Imaging, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, No. 141, Tianjin Road, Huangshigang District, Huangshi, Hubei, P. R. China

E-mail: 17764246156@163.com

© The Authors 2026. This is an **open access** article under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

stem cells; MTX, methotrexate; MUT, mutant-type; OS, osteosarcoma; PDCD4, programmed cell death 4; PDGF, platelet-derived growth factor; PMVs, platelet-derived microvesicles; PRP, platelet-rich plasma; RT, room temperature; SF serum-free; TEPs, tumor-educated platelets; 3'-UTR: 3'-untranslated region.

Introduction

There is a significant increasing trend in the incidence of malignant neoplasms of bone and articular cartilage (MNBAC) worldwide. In 2021, 91,375.1 (73,780.4–102,469.7) incident cases of MNBAC were predicted, and the number of deaths was 66,114.3 (53,305.4–74,466.9) (Lai et al. 2021). Recently, a global burden of disease (GBD) analysis indicated that adolescent and elderly populations share the heaviest disease burden of MNBAC (Zeng et al. 2025). Osteosarcoma (OS), chondrosarcoma, Ewing's sarcoma, and chordoma account for most MNBAC cases. The incidence in children under 15 years of age is 5.5 *per* million, with OS (2.8) and Ewing sarcoma (2.6) accounting for the majority (Kaatsch et al. 2016). Among multiple bone cancers, OS is one of the most common primary bone tumors among teenagers. In a recent retrospective analysis of 3446 OS patients, 26.9% were stage IIA, 38.0% were stage IIB, and 20.6% were stage III according to the Enneking staging system. Three percent of patients had nodal metastasis, and 19% had distant metastasis (Ogura et al. 2025). OS has a high incidence of lung metastases in patients, which occur in approximately 15–20% of osteosarcoma patients at diagnosis, considerably affecting the 5-year survival rate of patients (Luo et al. 2025). Resection and adjuvant chemotherapy are commonly used therapeutic methods in the clinic and have increased the overall 5-year survival rate of nonmetastatic patients to 60–70% (Mirabello et al. 2009). Complete surgical resection for OS remains essential for patient survival. However, excessive estimation of the tumor margin results in unwarranted tissue excision and substantial functional impairment postsurgery, whereas inadequate assessment of the boundary may lead to tumor recurrence (Meltzer et al. 2021; Zeng et al. 2024). The most effective regimens include the combination of high-dose methotrexate, doxorubicin, and cisplatin. However, the combination of other biologic agents or cytotoxic chemotherapy agents cannot definitively improve the survival of OS patients (Isakoff et al. 2015). Overall, 30–50% of OS patients experience recurrence, mostly 2–3 years after prospective treatment, with a recurrence rate of less than 30% (Whelan et al. 2018). Patients can survive for more than five years, and the 5-year survival rate after metastasis is less than 20% (Shoaib et al. 2022). Therefore, there is an urgent need to develop new treatment strategies to reduce metastasis in OS patients and improve overall survival rates.

Platelets are multipotent cells that not only play a role in hemostasis/coagulation and the inflammatory response but

also promote the malignant progression of tumors (Franco et al. 2015). During the progression of OS, platelets can protect tumor cells from shear forces and immune cell attacks, improving the survival rate of tumors during metastasis (Shi et al. 2024). For example, platelets undergo significant activation and release large amounts of lysophosphatidic acid (LPA) under the induction of OS. LPA or platelet-released fluid can cause morphological changes and increase the invasiveness of OS cells (Takagi et al. 2021). After the interaction between platelets and OS cells, platelets release platelet-derived growth factor (PDGF), which can promote the proliferation of OS cells (Takagi et al. 2014). Therefore, the interaction between platelets and OS cells has become an important factor promoting the malignant progression of tumors. However, the mechanism of platelet involvement in OS metastasis needs to be further explored.

MicroRNAs (miRNAs) are a type of endogenous non-coding RNA widely present in eukaryotes that can regulate gene expression and are approximately 21 to 23 nucleotides in length. By interacting with complementary sequences in the 3'-untranslated region (3'-UTR) of target gene mRNAs, mRNA attenuation and translation inhibition are induced (Ali et al. 2020). miRNAs can regulate tumor progression by modulating tumor proliferation, metastasis, angiogenesis, stem cell characteristics, and chemotherapy resistance (Bertoli et al. 2015). Platelets contain various nucleic acids, such as miRNAs, long noncoding RNAs (lncRNAs), and messenger RNAs (mRNAs). Platelet microbubbles contain fully functional miRNA processors that can transport their miRNAs to neighboring cells and regulate their gene expression (Leng et al. 2022). Interestingly, platelet miRNA profiles in cancer patients are abnormal and tumor specific. miRNAs enriched in platelets can regulate tumor proliferation, metastasis, tumor-associated immunity, tumor-associated thrombosis, and antitumor therapy. Therefore, miRNAs derived from platelets can directly affect tumor progression (Miao et al. 2021). miR-21 is one of the earliest discovered microRNAs and is located at the vacuolar membrane protein 1 locus on chromosome 17 (Lagos-Quintana et al. 2001). Interestingly, the levels of extracellular vesicle miR-21 in human serum and plasma are altered in OS patients compared with healthy people (Wang et al. 2020). miR-21 is upregulated in OS and promotes its progression (Ziyan et al. 2011; Li et al. 2021). Hence, platelet miR-21 may play an important role in OS progression.

On the basis of previous findings, we speculate that miRNAs derived from platelets are key molecules involved in OS progression. In this study, we found a considerable increase

in the number of platelet-derived microvesicles (PMVs) in OS patients (OS-PMVs). OS-PMVs enhanced the proliferation and metastasis ability of OS cells. We speculate that miR-21 derived from platelets promotes the progression of OS and affects its sensitivity to chemotherapy. Furthermore, the molecular mechanism of miR-21 was investigated. In summary, this study could provide new diagnostic and therapeutic targets for OS.

Materials and Methods

Clinical samples

For this study, we enrolled a total of 30 children and adolescent patients diagnosed with OS at Huangshi Central Hospital from 1 January 2022 to 30 June 2022. All patients were diagnosed for the first time through imaging, pathology, and other examinations. All patients had normal liver and kidney function, no other malignant lesions, and no preoperative radiotherapy or chemotherapy. Ten age- and sex-matched healthy individuals were recruited as the healthy control group. The baseline characteristics of the patients are shown in Table 1. The 30 OS patients included 17 males (56.7%) and 13 females (43.3%). The average age was 16.6 ± 3.8 years, and the median maximum tumor diameter was 15.2 cm. According to the Enneking surgical staging criteria, 19 patients (63.3%) were in stages I–II, and 11 patients (36.7%) were in stage III. Distant metastasis occurred in 13 patients (43.3%). Before any treatment, a sample of 5 ml of venous blood was taken and promptly spun at $1500 \times g$ for 15 min at 4°C . The resulting serum was then preserved in 1.5-ml RNase-free tubes at -80°C . The isolation of platelets and PMVs was performed according to a previous study (Tutuian et al. 2024). Briefly, platelet-rich plasma was isolated from 5 ml of whole blood by centrifugation ($200 \times g$, 20 min) at room temperature (RT). Washed platelets were isolated from the platelet-rich plasma (PRP) by centrifugation ($800 \times g$, 12 min) at RT. The platelets were subsequently resuspended in Tyrode's buffer (0.5 mM MgCl_2 , 5.6 mM glucose, 5 mM HEPES, 0.55 mM NaH_2PO_4 , 7 mM NaHCO_3 , 150 mM NaCl, and 2.7 mM KCl). Following activation with thrombin (0.1 U/ml) for 60 min, the platelets were pelleted *via* centrifugation at $3200 \times g$ (22°C , 10 min), and the PMVs were isolated *via* centrifugation at $20,000 \times g$ for 90 min at 18°C and then resuspended in Tyrode's buffer. Approval for this study was granted by the Ethics Committee at Huangshi Central Hospital (Approval No. Lunkuaishen (2023) Ke No. 3). The experiments were conducted in strict adherence to the Helsinki Declaration.

Cell culture

MG63 (No. CRL-1427) and U2OS (No. HTB-96), two human OS cell lines collected from the ATCC, were purchased

from Wuhan Procell Life Technology Co., Ltd. (Wuhan, China). The Cat. No. of MG63 is CL-0157, and the Cat. No. of U2OS is CL-0236. MG63 cells were cultured in MEM (PM150410B, Wuhan Procell, China) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, Carlsbad, USA). U2OS cells were cultured in McCoy's 5A medium (CM-0236, Wuhan Procell, China) supplemented with 10% FBS (Invitrogen, Carlsbad, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, Carlsbad, USA). The two cell lines were then cultured in a constant-temperature incubator at 37°C , 5% CO_2 , and saturated humidity.

Cell transfection

MG63 and U2OS cells in the logarithmic growth phase were dispersed in a 6-well plate (5×10^6 cells/well). After stable growth, miR-21 mimics (miR1000076-1-5, RIBOBIO, Guangzhou, China) or miR-21 inhibitors (miR20000076-1-5, RIBOBIO, Guangzhou, China) were transfected into MG63 cells *via* LipofectamineTM 2000 (11668030, Thermo, Waltham, US) according to the manufacturer's instructions. Twenty-four hours after transfection, the cells were collected. qRT-PCR was conducted to detect the miR-21 level, and the transfection efficiency was confirmed.

Cell treatment

MG63 and U2OS cells were transfected with a miR-21 inhibitor, treated with serum-free (SF) media plus vehicle (PBS), SF media plus PMVs from healthy donors (H-PMVs), or SF media plus OS-PMVs, and then inoculated into a 96-well plate (1×10^3 /well). Increasing concentrations of MTX (0, 100, 200, 400, 800, and 1600 ng/ml) (Luo et al. 2023), ADM (0, 1.25, 2.5, 5, 10, and 20 μM) (Salzillo et al. 2021), and DDP (0, 3.75, 7.5, 15, 30, and 60 μM) (Zheng et al. 2024) were added for treatment. The drug sources used were as follows: cisplatin (DDP) (HY-17394, MCE, NJ, US), methotrexate (MTX) (HY-14519, MCE, NJ, US), and doxorubicin (ADM) (HY-15142, MCE, NJ, US).

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

Total RNA was extracted from MG63 cells using TRIzol reagent (15596026CN, Thermo, Waltham, US), and its purity and concentration were detected. For microRNA qRT-PCR, a miRScript II RT kit (Qiagen) was used for reverse transcription, and a miRScript SYBR Green PCR kit (Qiagen) and a QuantStudio 6 Flex PCR instrument (Thermo Fisher Scientific, MA) were used for qRT-PCR. For the qRT-PCR of PDCD4, cDNA reverse transcription was performed using an iScript cDNA synthesis kit (Bio-Rad), and SsoAdvanced

Universal SYBR Green Super Mix (Bio-Rad) was used for qRT-PCR. The internal reference gene of PDCD4 was GAPDH. The serum miR-21 levels were normalized to those of U6. The relative expression level was calculated using the $2^{-\Delta\Delta C_t}$ method, and the experiment was repeated three times. The sequences of the primers used were as follows: PDCD4: positive, 5'-CGACAGTGGAGTGACGCCCTTA-3'; reverse, 5'-CGACACCTTGCCTCCTGACC-3'; GAPDH: positive, 5'-GCACGTCAAGTGAGAAC-3'; reverse, 5'-TGGTGAA-GACGCAGTGGGA-3'; miR-21: 5'-GTGCAGGGTCCGAG-GT-3'; reverse, 5'-GCCGTAGCTTTATCAGACTGATGT-3'; U6: positive, 5'-TGCGGGGTGCTCGCTTCGCGC-3'; reverse, 5'-CCAGTGCAGGGTCCGAGGT-3'.

Cell Counting Kit-8 (CCK8) assay

MG63 and U2OS cells were seeded into a 96-well plate (5×10^3 /well) and subsequently cultured. The cells were mixed with 10 μ l of CCK-8 solution (CK04, Dojindo, Kumamoto, Japan) at 24 h, 48 h, and 72 h and then incubated at 37°C for 2 h. A microplate reader (A51119500C, Thermo, Waltham, US) was used to measure the absorbance at 450 nm.

EdU staining assay

A BeyoClick™ EdU- 488 Cell Proliferation Detection Kit (C0071S, Beyotime, Shanghai, China) was used to detect cell proliferation. EdU (10 mM) was diluted with cell culture medium (1:500) to obtain the 2X EdU working solution (20 μ M). Then, the preheated 2X EdU working solution (20 μ M) was mixed at 37°C with the culture medium at a 1:1 ratio and cocultured for 2 h in a 6-well plate. The cells were fixed, washed, and permeabilized according to the instructions. Then, Hoechst 33342 (C1029, Beyotime, Shanghai, China) was used for nuclear staining for 5 min. The cells were observed and counted using a fluorescence microscope (Evos Flauto, Life Technologies, USA), and cell counting was performed *via* ImageJ software.

Transwell assay

MG63 and U2OS cells were digested and dispersed with 0.25% trypsin (40126ES60, Yisheng Biotechnology (Shanghai) Co., Ltd., Shanghai, China). The cells were subsequently resuspended in serum-free MEM, and 1×10^5 cells *per* well were inoculated into the upper chamber of a Transwell system (3464, 8 μ m, 24-well format; Corning, New York, US). Next, 500 μ l of culture medium containing 10% FBS was added to the lower chamber. After incubation at 37°C for 24 h, the cells that failed to migrate from the upper chamber were removed. Transwell membranes were fixed at room temperature with 4% paraformaldehyde (P1110, Solarbio,

Beijing, China) for 10–15 min and then stained with 0.5% crystal violet dye (C8470, Solarbio, Beijing, China) for 15 min. After the samples were rinsed with tap water, they were counted under a microscope. All the experiments were conducted in triplicate and repeated three times.

Colony formation assay

MG63 and U2OS cells in the logarithmic growth phase were digested with 0.25% trypsin (40126ES60, Yisheng Biotechnology Co., Ltd., Shanghai, China), and the resulting cell suspension was seeded into a 6-well plate with 1000 cells *per* well. After the cells were spread evenly, they were cultured at 37°C in a 5% CO₂ incubator until visible clones formed. The medium was changed every 3 days during this period. After 10 days, the cells were washed three times with phosphate-buffered saline (PBS) (ST447-1 L; Beyotime, Shanghai, China) and then fixed with 4% paraformaldehyde (P1110; Solarbio, Beijing, China) for 30 min. After the samples were washed again, they were stained with 0.5% crystal violet dye (C8470, Solarbio, Beijing, China) for 15 min. The samples were photographed and counted after washing and drying.

Western blotting

After cell collection *via* low-speed centrifugation, the cells were lysed with RIPA buffer (P0013B, Beyotime, Shanghai, China) supplemented with 1% PMSF, and the total protein was extracted. A Bradford protein concentration assay kit (P0006, Beyotime, Shanghai, China) was used to measure the protein concentration. Equal-quality proteins were separated *via* SDS-PAGE (Hefei, China, Delf-18044), and the separated proteins were transferred to PVDF membranes, which were then blocked with 5% skim milk at room temperature for 1 h. The membranes were incubated with an anti-PDCD4 antibody (1:500, A01105-3) or an anti-GAPDH antibody (1:2000, BM3874) purchased from Wuhan Doctoral Institute. The membranes were incubated overnight at 4°C. The membranes were washed 3–5 times with TBST buffer (T1081, Solarbio, Beijing, China) and then incubated with an HRP-labeled anti-rabbit IgG (1:2000, BA1061) purchased from Wuhan Doctoral. The mixture was incubated at room temperature for 1 h. After the membranes were washed three times, the protein bands were visualized using an enhanced chemiluminescence (ECL) kit (Millipore, Bedford, MA, USA) and imaged using a scanning instrument.

Dual-luciferase reporter gene assay

To verify the direct binding of miR-21 to PDCD4 mRNA, a dual-luciferase reporter gene assay was performed. The wild-type (WT) and mutant (MUT) binding sites of miR-21 in the PDCD4 3'-UTR sequence were inserted into the pmir-

GLO dual-luciferase vector, and the insertion was confirmed by sequencing. PDCD4-WT or PDCD4-MUT plasmids were transfected into MG63 cells along with miR-21 mimics or negative control miRNA. Forty-eight hours later, a dual-luciferase reporter gene assay was performed (Promega, WI, USA). All the experiments were conducted in triplicate and repeated three times.

Bioinformatic analysis

The LinkedOmics platform serves as an open-access repository encompassing multiomics datasets across all 32 cancer types documented in the TCGA project (Vasaikar et al. 2018). This online tool was employed to analyze miRNAs that are significantly associated with sarcoma histological subtypes. To identify osteosarcoma-related overall survival (OS)-associated genes, the GeneCards database was queried using “osteosarcoma” as the search term, with protein-coding genes scoring > 2 included in the analysis (Stelzer et al. 2016). The Gene Expression Omnibus (GEO) functions as an international public archive for high-throughput microarray and next-generation sequencing functional genomic datasets contributed by the research community. ENCORI (<https://rnasysu.com/encori/>), an extensive interactive atlas, integrates precise RNA interaction data identified *via* the innovative rbsSeeker and rriScan algorithms developed by Li et al. (2014), which were utilized to predict targets of miR-21. The Database for annotation, visualization, and integrated discovery (DAVID) (<https://david.ncifcrf.gov/home.jsp>) provides comprehensive functional annotation tools to decipher the biological significance of large gene lists (Sherman et al. 2022). Venn diagrams were used to intersect potential miR-21 target genes with OS-associated genes, and core targets were subjected to enrichment analysis *via* Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses using DAVID. The online K-M plotter tool (<https://kmplot.com/analysis/>) was used to assess overall survival outcomes in sarcoma patients (Győrffy et al. 2024).

Subcutaneous xenograft model in nude mice

BALB/c male nude mice, weighing 18–22 g and aged 6 weeks, were procured from Vitalstar Biotechnology Co., Ltd., located in Beijing, China. These mice were housed in an environment where the temperature was maintained at $22 \pm 2^\circ\text{C}$, the relative humidity was $55 \pm 5\%$, and a 12 h light/dark cycle was used. They were provided unrestricted access to sterile food and water. Following a 1-week acclimation period, the mice were randomly assigned to two groups of ten mice each, in accordance with the principle of complete randomization. MG63 cells were incubated in either serum-free (SF) medium or SF medium supplemented with OS-

PMVs for 24 h. Subsequently, $100\ \mu\text{l}$ of the cell suspension, containing approximately 5×10^6 cells, was injected into the right lateral hindlimb on the back of each nude mouse. The tumor volume was calculated using the following formula: $V = (\text{length} \times \text{width}^2)/2$. Thirty days after inoculation, the tumors were collected and weighed. The tumor tissues were then excised, measured for mass, and preserved. All animal experimental procedures were approved by the Ethics Committee of Huangshi Central Hospital (approval No. Lunkuaishen (2023) Ke No. 3).

Data analysis

Statistical calculations were carried out using SPSS (SPSS, Chicago, IL, USA) and GraphPad Prism version 8.10 (GraphPad Software, La Jolla, CA, USA). Categorical variables are displayed as percentages and were assessed using a chi-square test, Wilcoxon rank-sum test, and Fisher's exact probability. Differences in means were examined using Student's *t*-test or analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. A *p* value less than 0.05 was considered to indicate a significant difference.

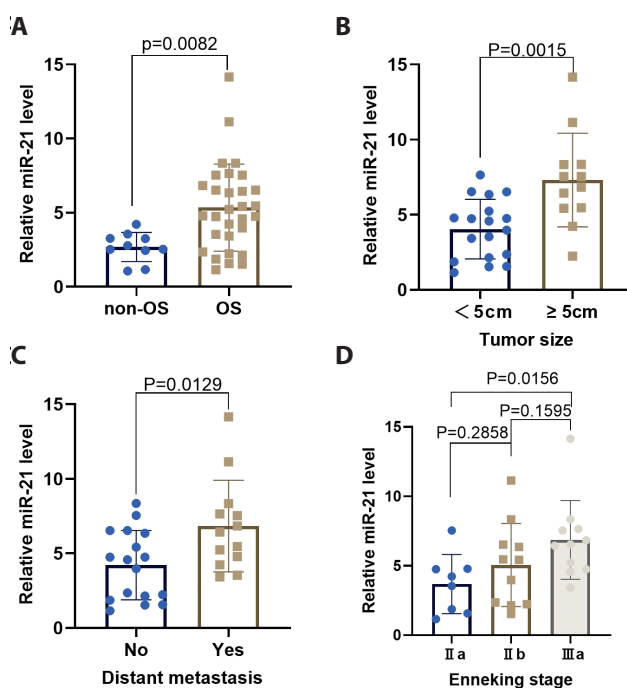
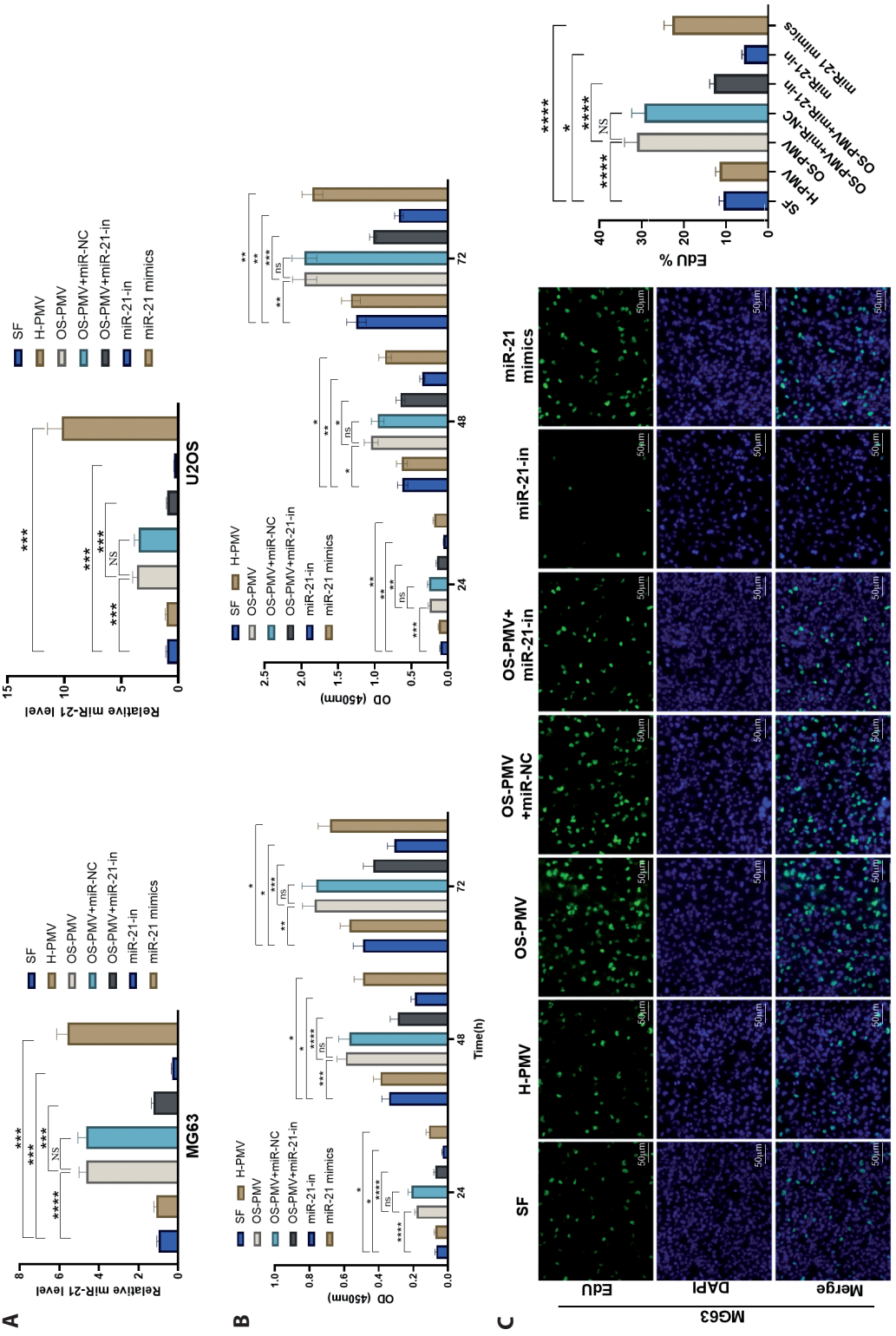


Figure 1. Altered miR-21 levels in OS patients. qRT-PCR was performed to determine the miR-21 level in the serum of OS patients. **A.** miR-21 levels in non-OS controls and OS patients. miR-21 alterations in OS patients with different tumor sizes (**B**), distant metastases (**C**), and differentiated states (**D**). Differences between means were analyzed by Student's *t* test. The error bars represent the standard deviation (SD).



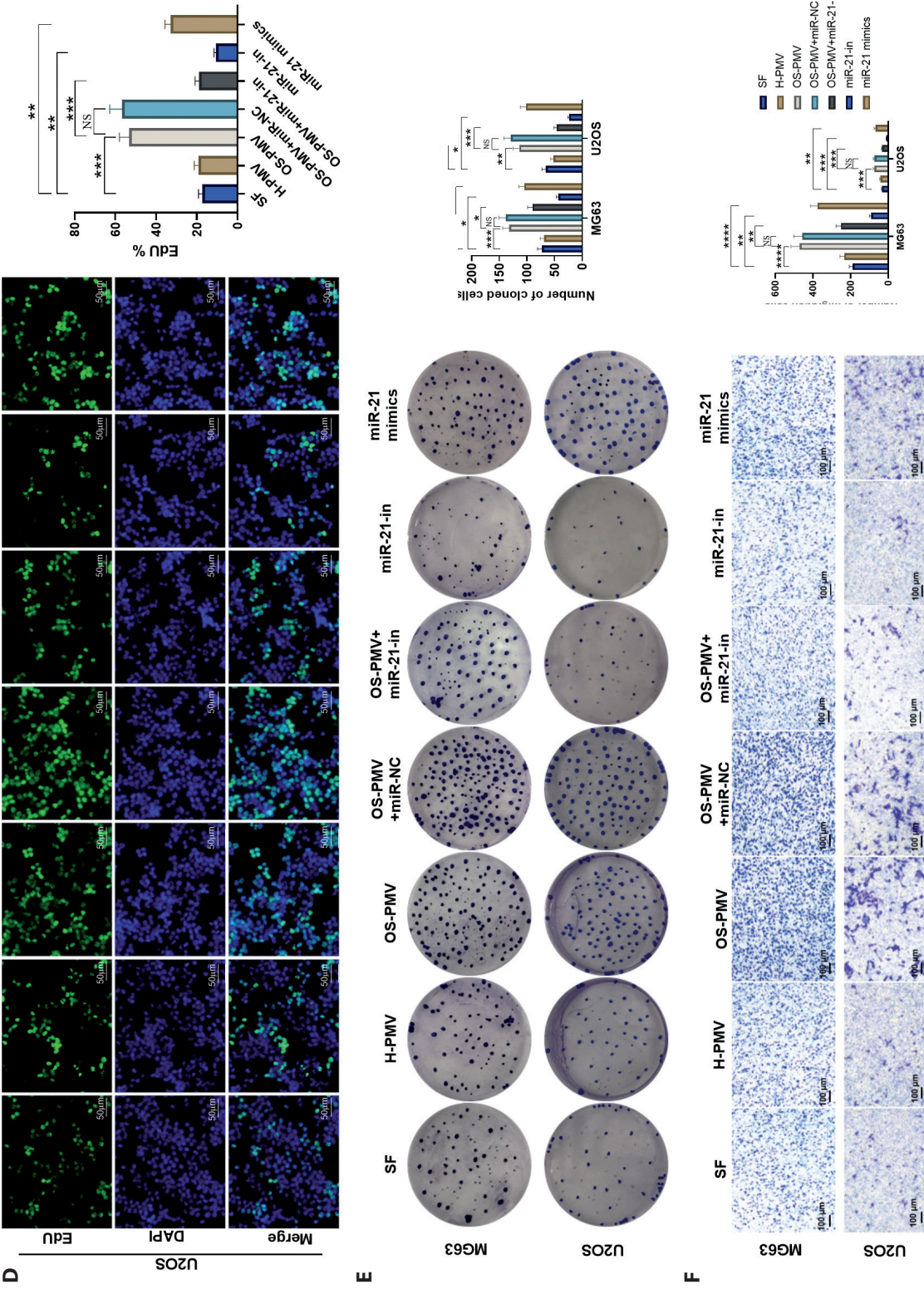


Figure 2. Role of miR-21 in OS-PMV-mediated oncogenic effects in OS cells. MG63 and U2OS cells were transfected with miR-21 inhibitors or mimics and then treated with H-PMVs or OS-PMVs. **A.** qRT-PCR was conducted to detect miR-21 in OS cells. **B.** A CCK8 assay was used to test the viability of OS cells. **C,D.** A EdU staining assay was carried out to detect cell proliferation and the percentage of EdU-positive cells was determined. Scale bar = 50 μ m. **E.** The colony formation assay was carried out and the number of cell colonies was counted. **F.** Transwell assays were used to assess the migratory ability of OS cells. All the experiments were biologically repeated three times ($n = 3$). The error bars represent the standard deviation (SD). Differences between means were analyzed by ANOVA followed by Tukey's *post hoc* test. NS $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

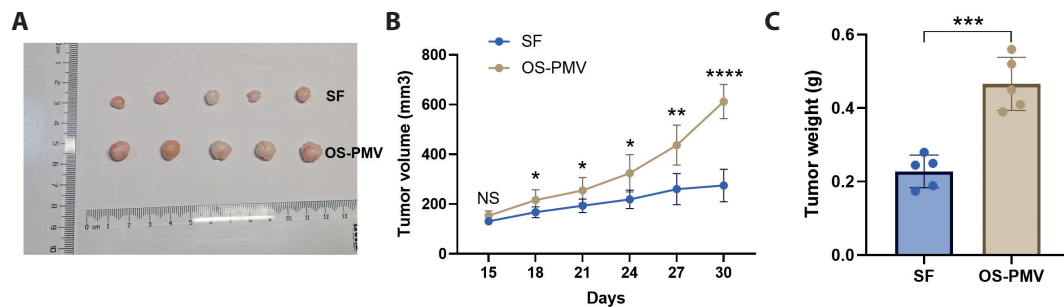


Figure 3. Role of OS-PMVs in the growth of MG63 cells *in vivo*. MG63 cells were incubated in either serum-free (SF) medium or SF medium supplemented with OS-PMVs for 24 h. One hundred microliters of the cell suspension (5×10^6 cells) was injected into the right lateral hindlimb on the back of each nude mouse. **A.** Tumor tissues were then excised 30 days later, and images of the tumors are shown. **B.** The tumor volume was calculated. **C.** Tumor weights were measured. Each group included 5 mice ($n = 5$). The error bars represent the standard deviation (SD). Differences between means were analyzed by ANOVA followed by Tukey's *post hoc* test. ^{NS} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

Results

Identification of platelet-associated miRNAs in OS patients

The online database LinkedOmics was used to analyze the miRNAs significantly associated with sarcoma histological types ($p < 0.01$) (Fig. S1A in Supplementary material). The differentially expressed (DE) miRNAs were analyzed in two GEO datasets, GSE28423 and GSE65071 (Fig. S1B,C). We identified PLT-associated miRNAs (PLT-miRs) *via* GeneCards using the search term “platelet miRNA”. Ten miRNAs were identified, as shown by the Venn diagram (Fig. S1D). The online K-M plotter tool (<https://kmplot.com/analysis/>) was used to analyze the overall survival of sarcoma patients with high or low levels of 10 miRNAs. The results revealed that several elevated miRNAs, including hsa-mir-21 ($p = 0.057$), hsa-mir-15a ($p = 0.075$), hsa-mir-182 ($p = 0.0043$), hsa-mir-181d ($p = 0.079$), hsa-mir-183 ($p = 0.0019$), hsa-mir-130b ($p = 0.00061$), hsa-mir-501 ($p = 0.025$), hsa-mir-361 ($p = 0.00053$) and hsa-mir-502 ($p = 0.13$), are associated with poor prognosis in sarcoma patients (Fig. S2A–D and 2SF–J). A decrease in hsa-mir-22 levels was correlated with poor prognosis in sarcoma patients ($p = 0.07$, Fig. S2E).

Expression features of miR-21 in OS patients

qRT-PCR was conducted to detect the miR-21 levels in the serum of OS patients. The results revealed that miR-21 levels in OS patients were significantly elevated compared with those in non-OS controls ($p = 0.0082$, Fig. 1A). Next, the miR-21 levels in OS patients with different clinical features were analyzed (Fig. 1A–F, Table 2). High or low miR-21 levels were defined by the median split. Patients with tumors larger than 5 cm had increased serum miR-21 levels ($p =$

0.0015, Fig. 1B). OS patients with distant metastasis ($p = 0.0129$, Fig. 1C) and higher Enneking stages also had higher miR-21 levels ($p = 0.0156$, IIa vs. IIIa, Fig. 1D). In addition, the miR-21 levels in OS patients with extremities or nonextremities ($p = 0.5215$, Fig. S3A) or with poorly differentiated or well-differentiated states ($p = 0.1481$, Fig. S3B) were not significantly different.

miR-21 in OS-PMV-mediated oncogenic effects in OS cells

To confirm the effects of platelet-derived miR-21 on OS, MG63 and U2OS cells were transfected with miR-21 inhibitors or mimics and then treated with H-PMVs or OS-PMVs. qRT-PCR revealed that the miR-21 level was increased in MG63 and U2OS cells after OS-PMV treatment. The miR-21 inhibitor significantly attenuated the miR-21 level in OS cells (*vs.* the OS-PMV+miR-NC group, Fig. 2A,B). A CCK8 assay was used to test the viability of MG63 and U2OS cells. OS-PMV treatment increased cell viability, which was inhibited by miR-21 inhibitor transfection. In addition, miR-21 mimic transfection promoted cell viability, whereas the miR-21 inhibitor had the opposite effects (Fig. 2C,D). EdU staining and colony formation assays were performed to detect cell proliferation. The percentage of EdU-positive cells was significantly greater after OS-PMV treatment or miR-21 mimic transfection, whereas the miR-21 inhibitor mostly reversed the OS-PMV-mediated effects (Fig. 2E–I). The migration of OS cells was examined. OS-PMV treatment increased cell migration, and the miR-21 inhibitor attenuated cell migration (Fig. 2J–K). To further confirm the role of OS-PMVs in tumor growth, a subcutaneous xenograft model was established in nude mice. Compared with those in the SF group, the MG63 cells in the OS-PMV group had significant growth ability (Fig. 3A–C).

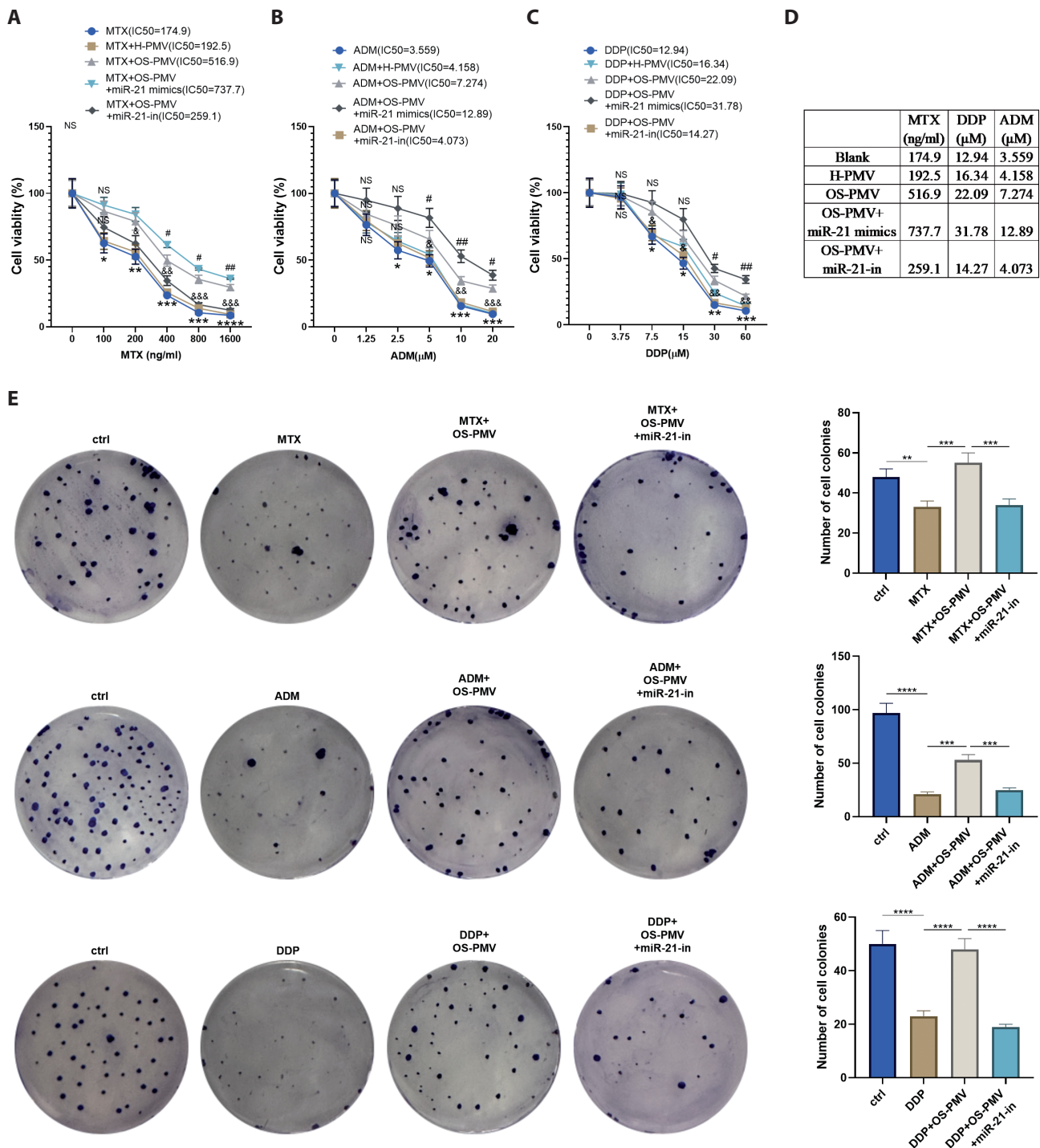


Figure 4. Effects of miR-21 and OS-PMVs on the chemical drug sensitivity of OS cells. MG63 OS cells were transfected with miR-21 inhibitors and then treated with OS-PMV and different doses of MTX (0–1600 ng/ml), ADM (0–20 μM), or DDP (0–60 μM). A–C. A CCK8 assay was used to detect cell viability. ^{NS} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. the MTX, ADM or DDP groups; ^{NS} $p > 0.05$, & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$, &&&& $p < 0.0001$ vs. the OS-PMV, ADM+OS-PMV or DDP+OS-PMV groups. D. The IC₅₀ values of these chemical drugs in MG63 cells were calculated. E. A colony formation assay was used to determine the colony formation ability of the cells. The number of cell colonies was counted. All the experiments were biologically repeated three times ($n = 3$). The error bars represent the standard deviation (SD). Differences between means were analyzed by Student's t test. The error bars represent the standard deviation (SD). ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

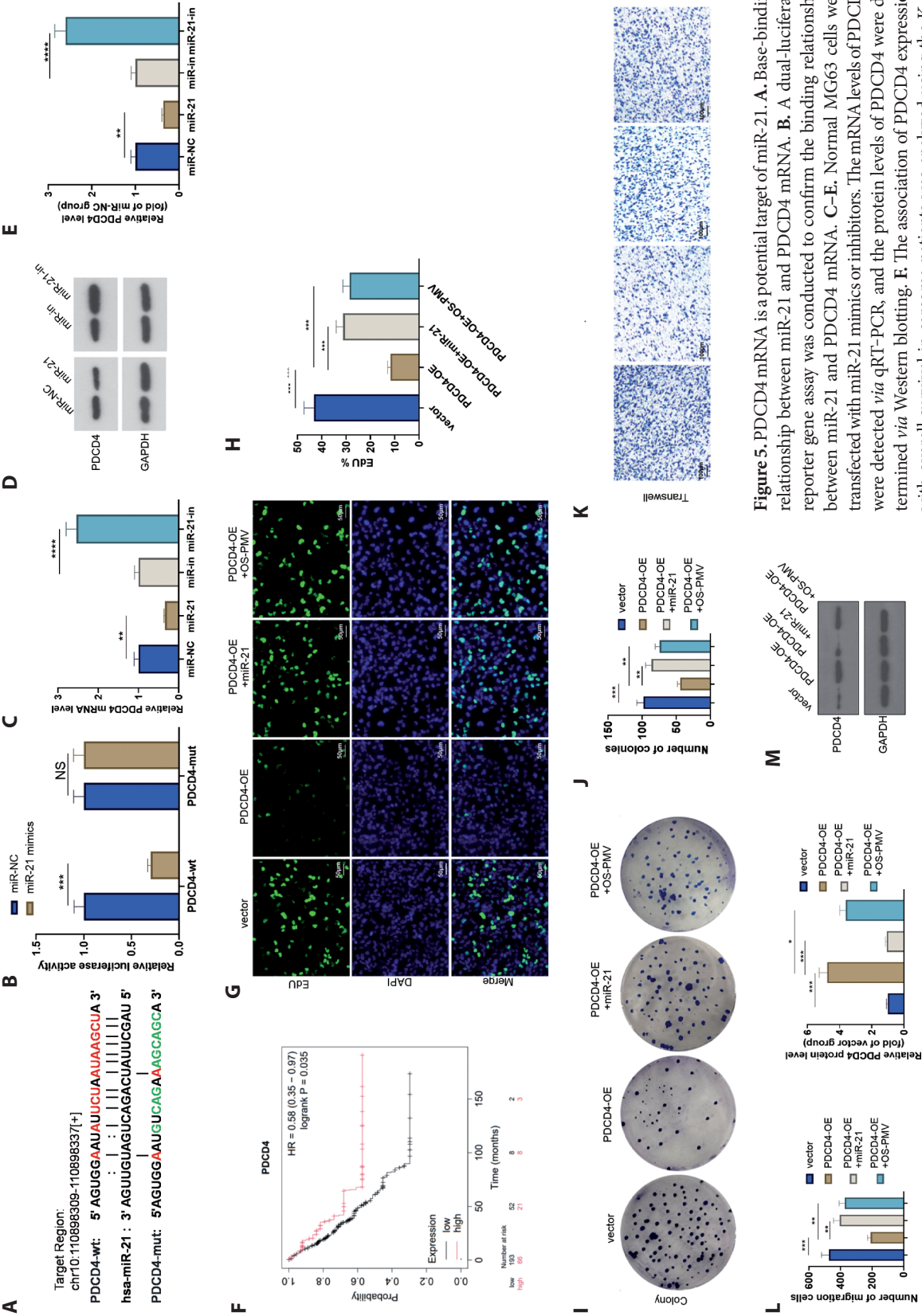


Figure 5. PDCD4 mRNA is a potential target of miR-21. **A.** Base-binding relationship between miR-21 and PDCD4 mRNA. **B.** A dual-luciferase reporter gene assay was conducted to confirm the binding relationship between miR-21 and PDCD4 mRNA. **C–E.** Normal MG63 cells were transfected with miR-21 mimics or inhibitors. The mRNA levels of PDCD4 were detected *via* qRT-PCR, and the protein levels of PDCD4 were determined *via* Western blotting. **F.** The association of PDCD4 expression with overall survival in sarcoma patients was analyzed using the K-M plotter (<https://kmplot.com/analysis/>). A PDCD4-overexpressing (PDCD4-OE) cell model was established in MG63 cells, followed by miR-21 mimic transfection or OS-PMV treatment. **G,H.** Edu staining and colony formation assays were carried out to detect cell proliferation. The percentage of Edu-positive cells was determined. Scale bar = 50 μ m. **I,J.** The number of cell colonies was counted. **K,L.** Transwell assays were used to assess the migratory ability of MG63 cells. **M.** The protein levels of PDCD4 were determined *via* Western blotting. All the experiments were biologically repeated three times ($n = 3$). The error bars represent the standard deviation (SD). NS $p > 0.05$, * $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

Inhibiting miR-21 enhances the chemical drug sensitivity of OS cells.

To test the effects of OS-PMVs on the chemosensitivity of OS cells, MG63 cells were transfected with miR-21 inhibitors or miR-21 mimics and then treated with OS-PMVs and various doses of MTX, ADM, or DDP. As shown by the results of the CCK8 assay, OS-PMVs significantly attenuated the MTX-, ADM- and DDP-induced decrease in cell viability and increased the IC50 of these chemical drugs. However, the miR-21 inhibitor reversed most OS-PMV-mediated effects (Fig. 4A-D). Compared with the MTX, ADM or DDP groups, the OS-PMV treatment group presented increased colony formation ability. Inhibiting miR-21 attenuated the colony formation ability of the cells (Fig. 4E).

Analysis of miR-21 targets in OS

The GeneCards website was used to analyze OS-associated genes. Those protein-coding genes with a score >2 were included. The miR-21 targets were analyzed using ENCORI. The Venn diagram revealed 83 common genes that might be targets of miR-21 (Fig. S4A). The miR-21-gene network is shown in Figure S4B. We conducted gene set enrichment analysis (GSEA) and found that 83 genes were enriched in mediating multiple processes, such as focal adhesion (Fig. S4C). KEGG pathways, such as “microRNAs in cancer”, “proteoglycans in cancer”, and other cancer-associated pathways, were enriched (Fig. S4D).

miR-21 targeted and inhibited PDCD4 mRNA expression in MG63 cells.

As predicted by ENCORI (<https://rnasyu.com/encori/>), the base-binding relationships between miR-21 and PDCD4 mRNA are shown in Figure 5A. We conducted a dual-luciferase reporter gene assay to confirm the binding relationship between miR-21 and PDCD4 mRNA. The results revealed that the miR-21 mimics significantly attenuated the luciferase activity of MG63 cells transfected with PDCD4-wt, but no significant difference was detected in PDCD4-mut-transfected cells (Fig. 5B). MG63 cells were transfected with miR-21 mimics or inhibitors. The mRNA and protein levels of PDCD4 were detected. The results revealed that the miR-21 mimic significantly attenuated PDCD4 mRNA and protein expression, whereas the miR-21 inhibitor markedly promoted PDCD4 mRNA and protein expression (Fig. 5E). The association of PDCD4 levels with the overall survival of sarcoma patients was analyzed using K-M plotter (<https://kmplot.com/analysis/>), a public database, which revealed that lower PDCD4 levels were associated with poorer overall survival in sarcoma patients (Fig. 5F). Next, we conducted

functional assays to confirm the potential role of miR-21-PDCD4 in OS. A PDCD4-overexpressing (PDCD4-OE) cell model was established in MG63 cells, followed by miR-21 mimic transfection or OS-PMV treatment. Compared with the vector group, the PDCD4-OE group presented a lower percentage of EdU-positive cells (Fig. 5G), fewer cell colonies (Fig. 5I,J), and decreased cell migration ability (Fig. 5K,L). However, miR-21 mimics transfection, and OS-PMV treatment could reverse these repressive alterations (Fig. 5G-L). Western blotting data confirmed that the PDCD4-OE group had increased PDCD4 expression, miR-21 mimics transfection, and OS-PMV treatment contributed to reduced PDCD4 expression (Fig. 5M).

Discussion

Tumor-educated platelets (TEPs) are the main diagnostic markers for human cancers (Bravaccini et al. 2024; Karp et al. 2024). Platelets can ingest and release RNA, proteins, cytokines and growth factors. Thus, platelets can interact with the tumor microenvironment and mediate tumor progression (Mendoza-Almanza et al. 2020). Here, we found that the miR-21 level was greater in the serum of OS patients. Platelet-derived miR-21 from OS patients promoted the proliferation and migration of OS cells and attenuated the sensitivity of OS cells to chemical drugs. We believe that platelet-derived miR-21 is a novel diagnostic marker and therapeutic target in patients with OS.

Serum miRNAs are considered potential diagnostic biomarkers for OS (Gao et al. 2020). For example, Araki Y. and colleagues reported that several miRNAs, including miR-487b-3p, miR-146a-5p, miR-1260b, miR-1260a, and miR-4758-3p, are considerably elevated and that these miRNAs are associated with an increased survival rate in OS patients. In particular, higher serum miR-1260a levels can predict better overall survival, metastasis-free survival, and disease-free survival in OS patients (Araki et al. 2023). Chen et al. (2022) identified a group of 166 differentially expressed (DE) miRNAs in the serum of OS patients using the GEO database. Among these miRNAs, hsa-miR-326 and hsa-miR-338-3p are significantly associated with tumor-infiltrating immune cells and have high diagnostic potential for OS. Platelets are considered the major source of serum miRNAs, and miRNA profiles can reflect the conditions and functions of platelets (Wakabayashi et al. 2021; Wicik et al. 2022). Notably, platelet-derived miRNAs play essential roles in affecting tumor development. For example, miR-126-3p derived from platelets can enter tumor cells through microvesicles. miR-126-3p directly targets AKT2 and inhibits the proliferation and invasion of breast cancer cells, thus playing an antitumor role (Sibilano et al. 2022). Platelet particles contain high levels of miR-939, which

can be transported to ovarian cancer cells. miR-939 can promote the transition of the epithelial-like phenotype to the mesenchymal-like phenotype in ovarian cancer cells and promote the proliferation and migration of tumor cells (Tang et al. 2017).

miR-21 is a key oncogene that is highly expressed in most cancers. For example, overexpression of miR-21 can lead to a pre-B malignant lymphoid phenotype, and when miR-21 is inactivated, the tumor completely regresses within a few days, partially due to apoptosis (Medina et al. 2010). Compared with MCF-7 and SK-BR-3 breast cancer cells, MDA-MB-231 cells presented the highest expression level of miR-21. After miR-21 was knocked out, the epithelial-mesenchymal transition (EMT) in MDA-MB-231 cells was downregulated. Wnt-11 is a key target of miR-21, and the Wnt-11-related signaling axis is altered in stable miR-21 knockout cells (Arisan et al. 2021). Studies have shown that extracellular vesicles derived from platelets are rich in miR-21 (Liao et al. 2023). After incubation with platelet-rich plasma (PRP), the osteogenic differentiation of mesenchymal stem cells (MSCs) is enhanced, accompanied by a significant increase in miR-21 levels, indicating that miR-21 derived from platelets is involved in MSC differentiation (Darabi et al. 2023). In this study, we found that serum miR-21 levels are significantly higher in OS patients than in healthy controls. Higher miR-21 levels were found in OS patients with larger tumor sizes, distant metastasis, and higher Enneking stages. These results indicate that serum miR-21 is a potential diagnostic marker in OS.

Circulating miRNAs can directly regulate the malignancy of tumor cells (Wu et al. 2024). For example, decreased serum miR-491 levels are associated with poorer clinical outcomes in OS patients, as reflected by lung metastasis, poorer chemotherapy responses, and a lower survival rate. miR-491 suppression contributes to aggravated lung metastasis and DDP resistance in OS cells (Wang et al. 2017). High levels of exosomal miR-184 and miR-3913-5p in the peripheral blood of non-small cell lung cancer patients can predict osimertinib resistance (Li et al. 2021). In this study, we found that OS-PMVs could promote the proliferation and migration of OS cells by delivering miR-21 into tumor cells. Moreover, OS cells treated with OS-PMVs presented reduced sensitivity to MTX, ADM, and DDP, the three major chemical drugs used to treat OS (Hattinger et al. 2021). However, inhibiting the miR-21 level can not only repress the proliferation and migration of OS cells but also partially reverse the OS-PMV-mediated decrease in chemical drug sensitivity. Thus, we believe that targeting serum miR-21 can reverse chemoresistance in OS patients.

Programmed cell death 4 (PDCD4) is a tumor suppressor gene involved in the regulation of cell proliferation, apoptosis, and metastasis (Luo et al. 2022; Liu et al. 2024). Dysregulation of PDCD4 has been implicated in OS, con-

tributing to tumor development, progression, and invasion (Yang et al. 2017; Zhang et al. 2017). Moreover, reduced PDCD4 expression can affect chemoresistance *via* multiple pathways. For example, PDCD4 plays a role in doxorubicin chemoresistance in breast cancer cells by interacting with eukaryotic initiation factor-4A (eIF4A) (González-Ortiz et al. 2022). Interestingly, several studies have revealed that miRNAs can affect the development of cancers by targeting PDCD4. For example, oral squamous cell carcinoma (OSCC) cells with chemoresistance can produce exosomes enriched with miR-21, which can decrease DNA damage signaling in response to cisplatin. PDCD4 has been identified as a direct target of miR-21 (Liu et al. 2017). In this study, we found that PDCD4 is not only a therapeutic target in OS but also a direct target of miR-21. Lower levels of PDCD4 can predict poorer overall survival in sarcoma patients. MiR-21 inhibits PDCD4 expression by directing the 3'-UTR of PDCD4 mRNA. Moreover, functional assays confirmed that PDCD4 overexpression inhibited the proliferation and migration of OS cells, and these effects were significantly reversed by miR-21 mimics. Therefore, platelet-derived miR-21 likely affects the development of OS by targeting PDCD4.

Conclusion

In summary, the present study revealed that serum miR-21 is a potential diagnostic marker for OS patients. Platelet-derived miR-21 can regulate the proliferation, metastasis, and chemotherapy resistance of OS cells. This study highlights the important role of platelet-derived miR-21 in diagnosing OS and enhancing the therapeutic effects of chemical drugs.

Authors contribution. XP, SD, FS: conceptualization, writing-review and editing; XP: methodology and data curation; SD, FS: validation; YZ: formal analysis; XP, JL, YZ: investigation; XP, JL, FS: resources; JL, FS, YZ: writing-original draft preparation; JL, BL: visualization and project administration; XP, SD: supervision; BL: funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflict of interests. The authors claim that there is no conflict of interest.

Funding information. This study was funded by the Natural Science Foundation of Hubei Province (No. 2023AFB1071).

Data availability statement. The data are available from the corresponding author on reasonable request.

Ethics approval and consent to participate. We obtained informed consent from patients before the study. Our study was approved by the Ethics Committee of the Huangshi Central Hospital (No. Lunkuaishen (2023) Ke No. 3).

References

- Ali Syeda Z, Langden SSS, Munkhzul C, Lee M, Song SJ (2020): Regulatory mechanism of microRNA expression in cancer. *Int. J. Mol. Sci.* **21**, 1723
<https://doi.org/10.3390/ijms21051723>
- Araki Y, Asano N, Yamamoto N, Hayashi K, Takeuchi A, Miwa Sh, Igarashi K, Higuchi T, Abe K, Taniguchi Y, et al. (2023): A validation study for the utility of serum microRNA as a diagnostic and prognostic marker in patients with osteosarcoma. *Oncol. Lett.* **25**, 222
<https://doi.org/10.3892/ol.2023.13808>
- Arisan ED, Rencuzogullari O, Cieza-Borrella C, Miralles Arenas F, Dwek M, Lange S, Uysal-Onganer P. (2021): miR-21 is required for the epithelial-mesenchymal transition in MDA-MB-231 breast cancer cells. *Int. J. Mol. Sci.* **22**, 1557
<https://doi.org/10.3390/ijms22041557>
- Bertoli G, Cava C, Castiglioni I. (2015): MicroRNAs: New biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. *Theranostics* **5**, 1122-1143
<https://doi.org/10.7150/thno.11543>
- Bravaccini S, Boldrin E, Gurioli G, Tedaldi G, Piano M A, Canale M, Curtarello M, Ulivi P, Pilati P (2024): The use of platelets as a clinical tool in oncology: opportunities and challenges. *Cancer Lett.* **607**, 217044
<https://doi.org/10.1016/j.canlet.2024.217044>
- Chen Z, Li L, Li Z, Wang X, Han M, Gao Z, Wang M, Hu G, Xie X, Duet H, et al. (2022): Identification of key serum biomarkers for the diagnosis and metastatic prediction of osteosarcoma by analysis of immune cell infiltration. *Cancer Cell. Int.* **22**, 78
<https://doi.org/10.1186/s12935-022-02500-6>
- Darabi MK, Pezeshki SP, Nazeri Z, Zarezadeh V, Dariuni HA, Kheirollah A, Galehdari H, Azizidoost S, Cheraghzadeh M (2023): Effect of platelet-rich plasma on the osteoblastic differentiation of human adipose tissue-derived mesenchymal stromal cells. *Clin. Lab.* **69**, 220224
<https://doi.org/10.7754/Clin.Lab.2022.221206>
- Franco AT, Corken A, Ware J (2015): Platelets at the interface of thrombosis, inflammation, and cancer. *Blood* **126**, 582-588
<https://doi.org/10.1182/blood-2014-08-531582>
- Gao SS, Wang YJ, Zhang GX, Zhang WT (2020): Potential diagnostic value of miRNAs in peripheral blood for osteosarcoma: A meta-analysis. *J. Bone Oncol.* **23**, 100307
<https://doi.org/10.1016/j.jbo.2020.100307>
- González-Ortiz A, Pulido-Capiz A, Castañeda-Sánchez CY, Ibarra-López E, Galindo-Hernández O, Calderón-Fernández MA, López-Cossio LY, Díaz-Molina R, Chimal-Vega B, Serafin-Higuera N, et al. (2022): eIF4A/PDCD4 pathway, a factor for doxorubicin chemoresistance in a triple-negative breast cancer cell model. *Cells* **11**, 4069
<https://doi.org/10.3390/cells11244069>
- Györfy B (2024): Integrated analysis of public datasets for the discovery and validation of survival-associated genes in solid tumors. *Innovation (Camb)* **5**, 100625
<https://doi.org/10.1016/j.xinn.2024.100625>
- Hattinger CM, Patrizio MP, Fantoni L, Casotti C, Riganti C, Serra M (2021): Drug resistance in osteosarcoma: emerging biomarkers, therapeutic targets and treatment strategies. *Cancers (Basel)* **13**, 2878
<https://doi.org/10.3390/cancers13122878>
- Isakoff MS, Bielack SS, Meltzer P, Gorlick R (2015): Osteosarcoma: current treatment and a collaborative pathway to success. *J. Clin. Oncol.* **33**, 3029-3035
<https://doi.org/10.1200/JCO.2014.59.4895>
- Kaatsch P, Strothotte J, Becker C, Bielack S, Dirksen U, Blettner M (2016): Pediatric bone tumors in Germany from 1987 to 2011: incidence rates, time trends and survival. *Acta Oncol.* **55**, 1145-1151
<https://doi.org/10.1080/0284186X.2016.1195509>
- Karp JM, Modrek AS, Ezhilarasan R, Zhang ZY, Ding Y, Graciani M, Sahimi A, Silvestro M, Chen T, Li S, et al. (2024): Deconvolution of the tumor-educated platelet transcriptome reveals activated platelet and inflammatory cell transcript signatures. *JCI Insight* **9**, e178719
<https://doi.org/10.1172/jci.insight.178719>
- Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001): Identification of novel genes coding for small expressed RNAs. *Science* **294**, 853-858
<https://doi.org/10.1126/science.1064921>
- Lai J, Li X, Liu W, Liufu Q, Zhong C (2024): Global, regional, and national burden and trends analysis of malignant neoplasm of bone and articular cartilage from 1990 to 2021. A systematic analysis for the global burden of disease study 2021. *Bone* **188**, 117212
<https://doi.org/10.1016/j.bone.2024.117212>
- Leng Q, Ding J, Dai M, Liu L, Fang Q, Wang DW, Wu L, Wang Y (2022): Insights into platelet-derived MicroRNAs in cardiovascular and oncologic diseases: potential predictor and therapeutic target. *Front. Cardiovasc. Med.* **9**, 879351
<https://doi.org/10.3389/fcvm.2022.879351>
- Li JH, Liu S, Zhou H, Qu LH, Yang JH (2014): starBase v2.0: Decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res.* **42**, D92-D97
<https://doi.org/10.1093/nar/gkt1248>
- Li LZ, Wu ZZ, Lv Z (2021): The clinical significance of mir-21 in guiding chemotherapy for patients with osteosarcoma. *Pharmgenomics Pers. Med.* **14**, 1247-1261
<https://doi.org/10.2147/PGPM.S321637>
- Li X, Chen C, Wang Z, Liu J, Sun W, Shen K, Lv Y, Zhu S, Zhan P, Lv T, Song Y, et al. (2021): Elevated exosome-derived miRNAs predict osimertinib resistance in non-small cell lung cancer. *Cancer Cell. Int.* **21**, 428
<https://doi.org/10.1186/s12935-021-02075-8>
- Liao TL, Liu HJ, Chen DY, Tang KT, Chen YM, Liu PY (2023): SARS-CoV-2 primed platelets-derived microRNAs enhance NETs formation by extracellular vesicle transmission and TLR7/8 activation. *Cell. Commun. Signal.* **21**, 304
<https://doi.org/10.1186/s12964-023-01345-4>
- Liu L, Feng X, Fan C, Kong D, Feng X, Sun C, Xu Y, Li B, Jiang Y, Zheng C, et al. (2024): PDCD4 interacting with PIK3CB and CTSZ promotes the apoptosis of multiple myeloma cells. *FASEB J.* **38**, e70024
<https://doi.org/10.1096/fj.202400687R>
- Liu T, Chen G, Sun D, Lei M, Li Y, Zhou C, Li X, Xue W, Wang H, Liu C, Xu J, et al. (2017): Exosomes containing miR-21 transfer

- the characteristic of cisplatin resistance by targeting PTEN and PDCD4 in oral squamous cell carcinoma. *Acta Biochim. Biophys. Sin. (Shanghai)* **49**, 808-816
<https://doi.org/10.1093/abbs/gmx078>
- Luo M, Deng S, Han T, Ou Y, Hu Y (2022): LncRNA NR2F2-AS1 functions as a tumor suppressor in gastric cancer through targeting miR-320b/PDCD4 pathway. *Histol. Histopathol.* **37**, 575-585
- Luo M, Su Z, Gao H, Tan J, Liao R, Yang J, Lin L (2023): Cirsiolol induces autophagy and mitochondrial apoptosis through the AKT/FOXO1 axis and influences methotrexate resistance in osteosarcoma. *J. Transl. Med.* **21**, 907
<https://doi.org/10.1186/s12967-023-04682-7>
- Luo Z, Ai T, Liu Z, He L, Hou Y, Li Y, Zhou Z, Shen X, et al. (2025): Predicting lung metastasis in high-grade Osteosarcoma: The role of CTA signs, with a focus on vascular wrapping and intra-tumoral vascular network. *J. Bone Oncol.* **52**, 100687
<https://doi.org/10.1016/j.jbo.2025.100687>
- Medina PP, Nolde M, Slack FJ (2010): OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. *Nature* **467**, 86-90
<https://doi.org/10.1038/nature09284>
- Meltzer PS, Helman LJ (2021): New horizons in the treatment of osteosarcoma. *N. Engl. J. Med.* **385**, 2066-2076
<https://doi.org/10.1056/NEJMra2103423>
- Mendoza-Almanza G, Burciaga-Hernández L, Maldonado V, Melendez-Zajgla J, Olmos J (2020): Role of platelets and breast cancer stem cells in metastasis. *World J. Stem. Cells* **12**, 1237-1254
<https://doi.org/10.4252/wjsc.v12.i11.1237>
- Miao S, Zhang Q, Chang W, Wang J (2021): New insights into platelet-enriched miRNAs: production, functions, roles in tumors, and potential targets for tumor diagnosis and treatment. *Mol. Cancer. Ther.* **20**, 1359-1366
<https://doi.org/10.1158/1535-7163.MCT-21-0050>
- Mirabello L, Troisi RJ, Savage SA (2009): Osteosarcoma incidence and survival rates from 1973 to 2004: data from the surveillance, epidemiology, and end results program. *Cancer* **115**, 1531-1543
<https://doi.org/10.1002/cncr.24121>
- Ogura K, Kawano H, Shimose S, Hiraoka K, Matsumine A, Kawai A (2025): Osteosarcoma in Japan: report from the bone and soft tissue tumor registry 2006-2022. *Jpn. J. Clin. Oncol.* **55**, 1046-1053
<https://doi.org/10.1093/jjco/hyaf094>
- Salzillo A, Ragone A, Spina A, Naviglio S, Sapio L (2021): Chlorogenic acid enhances doxorubicin-mediated cytotoxic effect in osteosarcoma cells. *Int. J. Mol. Sci.* **22**, 8586
<https://doi.org/10.3390/ijms22168586>
- Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T, Chang W, et al. (2022): DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res.* **50**, W216-W221
<https://doi.org/10.1093/nar/gkac194>
- Shi Q, Xu J, Chen C, Hu X, Wang B, Zeng F, Ren T, Huang Y, Guo W, Tang X, Ji T, et al. (2024): Direct contact between tumor cells and platelets initiates a FAK-dependent F3/TGF- β positive feedback loop that promotes tumor progression and EMT in osteosarcoma. *Cancer Lett.* **591**, 216902
<https://doi.org/10.1016/j.canlet.2024.216902>
- Shoaib Z, Fan TM, Irudayaraj JMK (2022): Osteosarcoma mechanobiology and therapeutic targets. *Br. J. Pharmacol.* **179**, 201-217
<https://doi.org/10.1111/bph.15713>
- Sibilano M, Tullio V, Adorno G, Savini I, Gasperi V, Catani MV (2022): Platelet-derived miR-126-3p Directly targets AKT2 and exerts anti-tumor effects in breast cancer cells: further insights in platelet-cancer interplay. *Int. J. Mol. Sci.* **23**, 5484
<https://doi.org/10.3390/ijms23105484>
- Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y, et al. (2016): The genecards suite: from gene data mining to disease genome sequence analyses. *Curr. Protoc. Bioinformatics* **54**, 1.30.1-1.30.33
<https://doi.org/10.1002/cpbi.5>
- Takagi S, Sasaki Y, Koike S, Takemoto A, Seto Y, Haraguchi M, Ukaji T, Kawaguchi T, Sugawara M, Saito M, et al. (2021): Platelet-derived lysophosphatidic acid mediated LPAR1 activation as a therapeutic target for osteosarcoma metastasis. *Oncogene* **40**, 5548-5558
<https://doi.org/10.1038/s41388-021-01956-6>
- Takagi S, Takemoto A, Takami M, Oh-Hara T, Fujita N (2014): Platelets promote osteosarcoma cell growth through activation of the platelet-derived growth factor receptor-Akt signaling axis. *Cancer Sci.* **105**, 983-988
<https://doi.org/10.1111/cas.12464>
- Tang M, Jiang L, Lin Y, Wu X, Wang K, He Q, Wang X, Li W (2017): Platelet microparticle-mediated transfer of miR-939 to epithelial ovarian cancer cells promotes epithelial to mesenchymal transition. *Oncotarget* **8**, 97464-97475
<https://doi.org/10.18632/oncotarget.22136>
- Tutuianu A, Anene CA, Shelton M, Speirs V, Whitelaw DC, Thorpe J, Roberts W, Boyne JR, et al. (2024): Platelet-derived microvesicles isolated from type-2 diabetes mellitus patients harbour an altered miRNA signature and drive MDA-MB-231 triple-negative breast cancer cell invasion. *PLoS One* **19**, e0304870
<https://doi.org/10.1371/journal.pone.0304870>
- Vasaikar SV, Straub P, Wang J, Zhang B (2018): LinkedOmics: analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Res.* **46**, D956-D963
<https://doi.org/10.1093/nar/gkx1090>
- Wakabayashi I, Sotoda Y, Eguchi R (2021): Contribution of platelet-derived microRNAs to serum microRNAs in healthy men. *Platelets* **32**, 984-987
<https://doi.org/10.1080/09537104.2020.1810223>
- Wang S, Ma F, Feng Y, Liu T, He S (2020): Role of exosomal miR 21 in the tumor microenvironment and osteosarcoma tumorigenesis and progression (Review). *Int. J. Oncol.* **56**, 1055-1063
<https://doi.org/10.3892/ijo.2020.4992>
- Wang SN, Luo S, Liu C, Piao Z, Gou W, Wang Y, Guan W, Li Q, Zou H, Yang Z, et al. (2017): miR-491 inhibits osteosarcoma lung metastasis and chemoresistance by targeting α -crystallin. *Mol. Ther.* **25**, 2140-2149
<https://doi.org/10.1016/j.ymthe.2017.05.018>
- Whelan JS, Davis LE (2018): Osteosarcoma, chondrosarcoma, and chordoma. *J. Clin. Oncol.* **36**, 188-193
<https://doi.org/10.1200/JCO.2017.75.1743>
- Wiczik Z, Czajka P, Eyileten C, Fitas A, Wolska M, Jakubik D, von Lewinski D, Sourij H, Siller-Matula JM, Postula M, et al. (2022):

- The role of miRNAs in regulation of platelet activity and related diseases – a bioinformatic analysis. *Platelets* **33**, 1052-1064
<https://doi.org/10.1080/09537104.2022.2042233>
- Wu Y, Zhang J, Lin F, Zhao Y, Zheng B, Zhou N, Zhang Z, Li G, Xie L, et al. (2024): Exosomal miR-1470 is a diagnostic biomarker and promotes cell proliferation and metastasis in colorectal cancer. *Cancer Med.* **13**, e7117
<https://doi.org/10.1002/cam4.7117>
- Yang C, Wang G, Yang J, Wang L (2017): Long noncoding RNA NBAT1 negatively modulates growth and metastasis of osteosarcoma cells through suppression of miR-21. *Am. J. Cancer. Res.* **7**, 2009-2019
- Zeng F, Li C, Wang H, Wang Y, Ren T, He F, Jiang J, Xu J, Wang B, Wu Y, et al. (2024): Intraoperative resection guidance and rapid pathological diagnosis of osteosarcoma using B7H3 targeted probe under NIR-II fluorescence imaging. *Adv. Sci. (Weinh)* **11**, e2310167
<https://doi.org/10.1002/adv.202310167>
- Zeng W, Yang W, Yu G, Shen P, Zheng Z, Yang F (2025): Burden and projections of malignant neoplasm of bone and articular cartilage in China: an analysis for the global burden of disease study 2021. *BMC Musculoskelet. Disord.* **26**, 512
<https://doi.org/10.1186/s12891-025-08760-3>
- Zhang R, Xia T (2017): Long non-coding RNA XIST regulates PDCD4 expression by interacting with miR-21-5p and inhibits osteosarcoma cell growth and metastasis. *Int. J. Oncol.* **51**, 1460-1470
<https://doi.org/10.3892/ijo.2017.4127>
- Zheng Z, Zeng Y, Bao X, Huang C, Guo F, Xu F, Luo Z (2024): OTULIN confers cisplatin resistance in osteosarcoma by mediating GPX4 protein homeostasis to evade the mitochondrial apoptotic pathway. *J. Exp. Clin. Cancer. Res.* **43**, 330
<https://doi.org/10.1186/s13046-024-03249-8>
- Ziyan W, Shuhua Y, Xiufang W, Xiaoyun L (2011): MicroRNA-21 is involved in osteosarcoma cell invasion and migration. *Med. Oncol.* **28**, 1469-1474
<https://doi.org/10.1007/s12032-010-9563-7>

Received: April 16, 2025

Final version accepted: July 15, 2025