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MTF2 facilitates the advancement of osteosarcoma through mediating EZH2/SFRP1/Wnt signaling

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Abstract

Background Osteosarcoma is a soft tissue neoplasm with elevated recurrence risk and highly metastatic potential. Metal response element binding transcriptional factor 2 (MTF2) has been revealed to exert multiple activities in human tissues. The present research was conducted to explore the functions and related response mechanism of MTF2 in osteosarcoma which have not been introduced yet.

Methods Bioinformatics tools identified the differential MTF2 expression in osteosarcoma tissues. MTF2 expression in osteosarcoma cells was examined with Western blot. Cell Counting Kit-8 (CCK-8) assay, 5-Ethynyl-2′-deoxyuridine (EDU) staining, wound healing as well as transwell assays measured cell proliferation, migration and invasion, respectively. Flow cytometry assay detected the cellular apoptotic level. Western blot also measured the expressions of proteins associated with epithelial mesenchymal transition (EMT), apoptosis and enhancer of zeste homolog 2 (EZH2)/secreted frizzled-related protein 1 (SFRP1)/Wnt signaling. Co-immunoprecipitation (Co-IP) assay confirmed MTF2-EZH2 interaction.

Results MTF2 expression was increased in osteosarcoma tissues and cells. MTF2 interference effectively inhibited the proliferation, migration and invasion of osteosarcoma cells and promoted the cellular apoptotic rate. MTF2 directly bound to EZH2 and MTF2 silence reduced EZH2 expression, activated SFRP1 expression and blocked Wnt signaling in osteosarcoma cells. EZH2 upregulation or SFRP1 antagonist WAY-316606 partly counteracted the impacts of MTF2 down-regulation on the SFRP1/Wnt signaling and the biological phenotypes of osteosarcoma cells.

Conclusions MTF2 might down-regulate SFRP1 to activate Wnt signaling and drive the progression of osteosarcoma via interaction with EZH2 protein.

Keywords Epithelial-mesenchymal transition, EZH2, Migration and invasion, MTF2, Osteosarcoma, SFRP1/Wnt signaling

Introduction

Osteosarcoma is a soft tissue neoplasm prevailing in the pediatric and adolescent age group (1). Osteosarcoma, which begins in osteoblast committed cells to produce osteoid or bone matrix and directly form immature bone or osteoid tissue, occurs frequently in long tubular bones such as the tibia and femur, resulting in swelling of the affected limb, limited joint movement, even fractures and preferentially metastasizing to the lung (2, 3). Despite the emergence of the standard treatment regimens for

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osteosarcoma, including wide local tumor excision and multiagent chemotherapy that have prolonged the life expectancy, the majority of patients still face dismal prognosis due to the high aggressiveness and metastasis, however (4, 5). Thereafter, it is essential to explore the hidden pathogenic mechanism of osteosarcoma for developing novel molecular markers.

As a catalytically inactive protein, metal response element binding transcriptional factor 2 (MTF2), also known as Polycomb-like 2 (PCL2), is a member of the PCL family possessing biological significance in cancers (6). As a well-known accessory protein of Polycomb Repressive Complex 2 (PRC2) which can catalyze H3K27 methylation, MTF2 can recruit PRC2 to the loci of target genes to enable embryonic stem cell differentiation via promoting H3K27me3 methylation and suppressing the pluripotency network (7–9). MTF2 expression is dysregulated in various human tissues and is increasingly recognized as a double-edged sword in human malignancies (10, 11). Particularly, previous research has mentioned that MTF2 expression is elevated in osteosarcoma tissues and cells and associated with the patients' outcome (12). Accordingly, to comprehensively specify the role of MTF2 in osteosarcoma, the detailed effects of MTF2 on the advancement of osteosarcoma demand to be affirmed.

According to the FpClass database, enhancer of zeste homolog 2 (EZH2) was predicated as a prospective interacting protein of MTF2. Intriguingly, as an H3K27me3 methyltransferase and a PRC2 catalytic subunit, EZH2 has been reported to be down-regulated in MTF2-knockout cells (13). Extensive literatures have well established the tumor-promoting role of EZH2 in osteosarcoma (14–17). Notably, secreted frizzled-related protein 1 (SFRP1) has been identified as a target gene of EZH2 (18–20). Furthermore, SFRP1 has been supported to play a tumor-suppressing role in osteosarcoma as an antagonist of oncogenic Wnt signaling (21, 22).

Combined with the above findings, the aim of the research was to investigate the effects of MTF2 on osteosarcoma and the relationship between MTF2 and EZH2/SFRP1/Wnt signaling.

Materials and methods

Bioinformatics tools

GSE14359 and GSE126209 are osteosarcoma-related datasets. Differentially expressed genes (DEGs) between 2 nonneoplastic primary osteoblasts and 18 conventional osteosarcoma samples in GSE14359 dataset, between 4 expression datasets of osteosarcoma tissues and 4 expression datasets of adjacent non-tumorous tissues in GSE126209 dataset were screened by GEO2R.

Culture and treatment of cells

Human fetal osteoblastic cell line (hFOB 1.19; RRID: CVCL_3708) and osteosarcoma cell lines MG-63 (RRID: CVCL_0426), HOS (RRID: CVCL_0312), U2OS (RRID: CVCL_0042) were all obtained from American Type Culture Collection (ATCC). hFOB 1.19 and U2OS cells were respectively incubated in Dulbecco's modified Eagle medium (DMEM)/F-12 (Chi Scientific, Jiangsu, China) and McCoy's 5a Medium (Chi Scientific, Jiangsu, China). The other osteosarcoma cell lines were incubated in Eagle's minimum essential medium (EMEM; Chi Scientific, Jiangsu, China). All media were supplemented with 10% fetal bovine serum (FBS; Chi Scientific, Jiangsu, China) and cell incubation was performed at 37 °C in a humidified incubator with 5% CO_2 except that hFOB 1.19 cell line was kept at a permissive temperature of 34 °C. Additionally, MG-63 cells were treated by SFRP1 antagonist WAY-316606 (25 μM; Cayman Chemical, Ann Arbor, MI, USA) for 24 h.

Gene interference and overexpression

The short hairpin RNA (shRNA) lentiviral particles against MTF2 (shRNA-MTF2-1: TCCCAATGAAAT GGTTATATG; shRNA-MTF2-2: ATGAAGGCAAAG AAGATTATC) were designed and produced by Sbo-Bio (Shanghai, China) with pLKO.1-puro vector as the backbone and the empty lentiviral vector (shRNA-NC) served as the negative control. The adenovirus vector of EZH2 (Ov-EZH2) was procured from Vigenebio (Shandong, China) using pcDNA3.1 vector as the backbone and the empty pcDNA3.1 vector served as the control (Ov-NC). These plasmids were transfected into MG-63 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Cell Counting Kit-8 (CCK-8)

Following the inoculation into the 96-well flat-bottomed plate (5×10^3 cells/well), the transfected MG-63 cells were exposed to 25 μM WAY-316606. Afterwards, 10 μl CCK-8 working liquid (DoGen, Daejeon, Korea) was supplemented to each well to incubate cells for 2 h. Under a microplate reader (Bio-TEK, Saxony, USA), the absorbance was detected at 450 nm.

5-Ethynyl-2′-deoxyuridine (EDU) staining

After being inoculated into 96-well plates (1.5×10^4 cells/well), the transfected MG-63 cells were exposed to 25 μM WAY-316606 and processed with EDU media (10 μM) for 1 h using EdU Labeling Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. After being exposed to fixative solution (Sangon Biotech, Shanghai, China) and the permeating solution (Sangon

Biotech, Shanghai, China), the cells were incubated with Click reaction solution (Sangon Biotech, Shanghai, China) and dyed by Hoechst 33,342 (Sangon Biotech, Shanghai, China). Under a fluorescence microscope (Olympus, Tokyo, Japan), the viable cells were observed.

Wound healing assay

After being inoculated into 6-well plates (5×10^5 cells/well), the transfected MG-63 cells were exposed to 25 μM WAY-316606. The confluent monolayer was then wounded by a micropipette tip to form the longitudinal cell-free regions. Under a light microscope (Olympus, Tokyo, Japan), the wound closure fraction was calculated at predetermined time intervals.

Transwell assay

After being exposed to 25 μM WAY-316606, the transfected MG-63 cells were suspended in FBS-deprived media and 500 μL medium containing 10% FBS and then respectively seeded into the upper and bottom transwell compartments (Millipore, Bedford, MA, USA) coated with Matrigel (BD Bioscience, San Jose, CA, USA). Cells that traversed the membrane filter to the lower surface were exposed to the fixative solution (Sangon Biotech, Shanghai, China) and dyed by 0.1% crystal violet (Sangon Biotech, Shanghai, China). The invaded cells were quantified under a light microscope (Olympus, Tokyo, Japan).

Flow cytometry assay

After digestion with EDTA-free pancreatin, the transfected MG-63 cells exposed to 25 μM WAY-316606 were combined with the cell supernatants and centrifuged at 500 xg for 5 min at 4 °C. After PBS washing, the cells were centrifuged at 500 xg for 5 min at 4 °C and resuspended in 1 × binding buffer to adjust the cell density as $1\sim5\times10^6$/mL. Subsequently, the cell suspension (100 μL) was processed with 5 μL Annexin V-FITC/PI (Servicebio, Wuhan, China) for 10 min at room temperature in the dark and incubated with 400 μL precooled 1 × binding buffer. Within 1 h, the cellular apoptotic level was detected using a flow cytometer (BD Bioscience, San Jose, CA, USA). The results were analyzed using FlowJo software (version 7.6.1; Tree Star, Inc., Ashland, OR, USA).

Co-immunoprecipitation (Co-IP)

Co-IP assay was conducted using a Co-IP kit (Servicebio, Wuhan, China) according to the manufacturer's instructions. Briefly, MG-63 cell lysates were incubated with MTF2 (cat. no. ab254336; 1/30; Abcam) and EZH2 (cat. no. ab307646; 1/30; Abcam) antibodies or horseradish peroxidase (HRP)-conjugated secondary antibodies (cat. no. ab205718; 1/30; Abcam). The protein A/G agarose beads (Santa Cruz Biotechnology, USA)-captured immune complexes separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE; Thermo Fisher Scientific, USA) were analyzed by Western blot.

Western blot

After cells were lysed with RadioImmunoPrecipitation Assay (RIPA) buffer containing 1% protease inhibitor (Aidlab, Beijing, China), the concentration of total protein was quantified using bicinchoninic acid (BCA) method (Aidlab, Beijing, China) according to the manufacturer's instructions. Then, the protein samples (30 μg/pore) were electrophoresed by 10% SDS-PAGE (Thermo Fisher Scientific, USA) and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA) which were sealed by 5% BSA (Millipore, Bedford, MA, USA). The membranes were then incubated with primary antibodies against MTF2 (cat. no. ab254336; 1/1000; Abcam), EZH2 (cat. no. ab307646; 1/1000; Abcam), E-cadherin (cat. no. #AF7718; 1/1000; Affinity Biosciences), N-cadherin (cat. no. #AF6710; 1/1000; Affinity Biosciences), Vimentin (cat. no. #AF7013; 1/1000; Affinity Biosciences), B cell lymphoma-2 (Bcl-2; cat. no. #AF0769; 1/1000; Affinity Biosciences), cleaved-caspase3 (cat. no. #AF7022; 1/1000; Affinity Biosciences), cleaved poly-ADP-ribose polymerases (PARP) (cat. no. #AF7023; 1/1000; Affinity Biosciences), SFRP1 (cat. no. #DF10172; 1/1000; Affinity Biosciences), Wnt3a (cat. no. #DF6113; 1/1000; Affinity Biosciences), β-catenin (cat. no. #AF6266; 1/1000; Affinity Biosciences) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; cat. no. #AF0911; 1/5000; Affinity Biosciences) overnight at 4 °C and HRP-conjugated secondary antibodies (cat. no. ab205718; 1/2000; Abcam) for 1 h and washed 3 times with 1 × TBST (Solarbio, Beijing, China). The protein bands were visualized by the ULTRAECL Substrate Chemiluminescence Detection Kit (Aidlab, Beijing, China) with the Electrophoresis Gel Imaging Analysis System (DNR Bio-Imaging Systems, Jerusalem, Israel). The protein bands were quantified using Image J software (version 1.46r; National Institutes of Health, Bethesda, MD, USA).

Statistical analyses

Data were analyzed using SPSS (version 22.0; IBM Corp., USA) and pictures were generated using GraphPad Prism 8.3.0 software (GraphPad Software, Inc., San Diego, CA, USA). All quantitative data were presented as mean ± standard deviation. The variations among multiple samples were determined by Student's t-test or analysis of variance (one-way) with Tukey's test. $P \leq 0.05$ indicated statistical significance.

Results

MTF2 expression is increased in osteosarcoma samples and cells

Based on the data from GSE14359 and GSE126209 dataset from GEO database, it was discovered that MTF2 expression was increased in osteosarcoma tissues compared with normal tissues (Fig. 1A). As presented in Fig. 1B, Western blot showed that MTF2 protein expression was higher in osteosarcoma cell lines including MG-63, HOS and U2OS than in human fetal osteoblastic cell line (hFOB 1.19), further suggesting the up-regulation of MTF2 in osteosarcoma. Therefore, MG-63 cells were adopted for following experiments because they had the highest MTF2 expression.

MTF2 silence inhibits the aggressive biological events of MG-63 cells

Following the transfection of MTF2 interference plasmids (shRNA-MTF2-1/2), MTF2 expression was successfully reduced in MG-63 cells (Fig. 2A). Moreover, MTF2 expression was lower in the shRNA-MTF2-1 group than in shRNA-MTF2-2 group, and then shRNA-MTF2-1 was selected for the following functional experiments. Results of CCK-8 assay revealed that the knockdown of MTF2 significantly reduced cell viability (Fig. 2B). Also, the data from EDU staining showed that cell proliferation was significantly inhibited when MTF2 was down-regulated (Fig. 2C). Consistently, after MTF2 was knocked down, the migration and invasion of MG-63 cells were both suppressed (Fig. 2D-E). EMT process is well documented as a central contributor to tumor metastasis. Through the detection of EMT markers, it was found that the inhibition of MTF2 increased E-cadherin expression

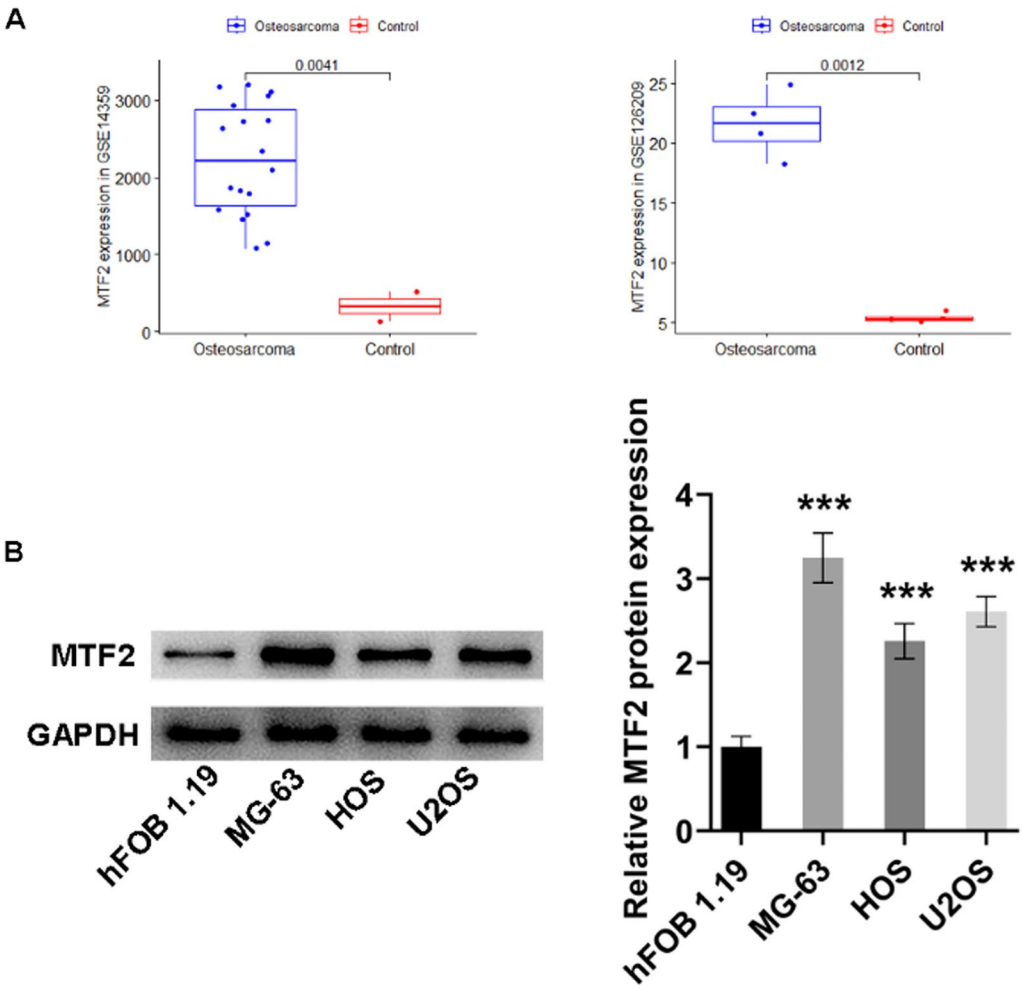

Fig. 1 MTF2 expression is increased in osteosarcoma samples and cells. **A** GSE14359 and GSE126209 dataset from TCGA database analyzed MTF2 expression in osteosarcoma tissues. **B** Examination of MTF2 expression by Western blot in osteosarcoma cells

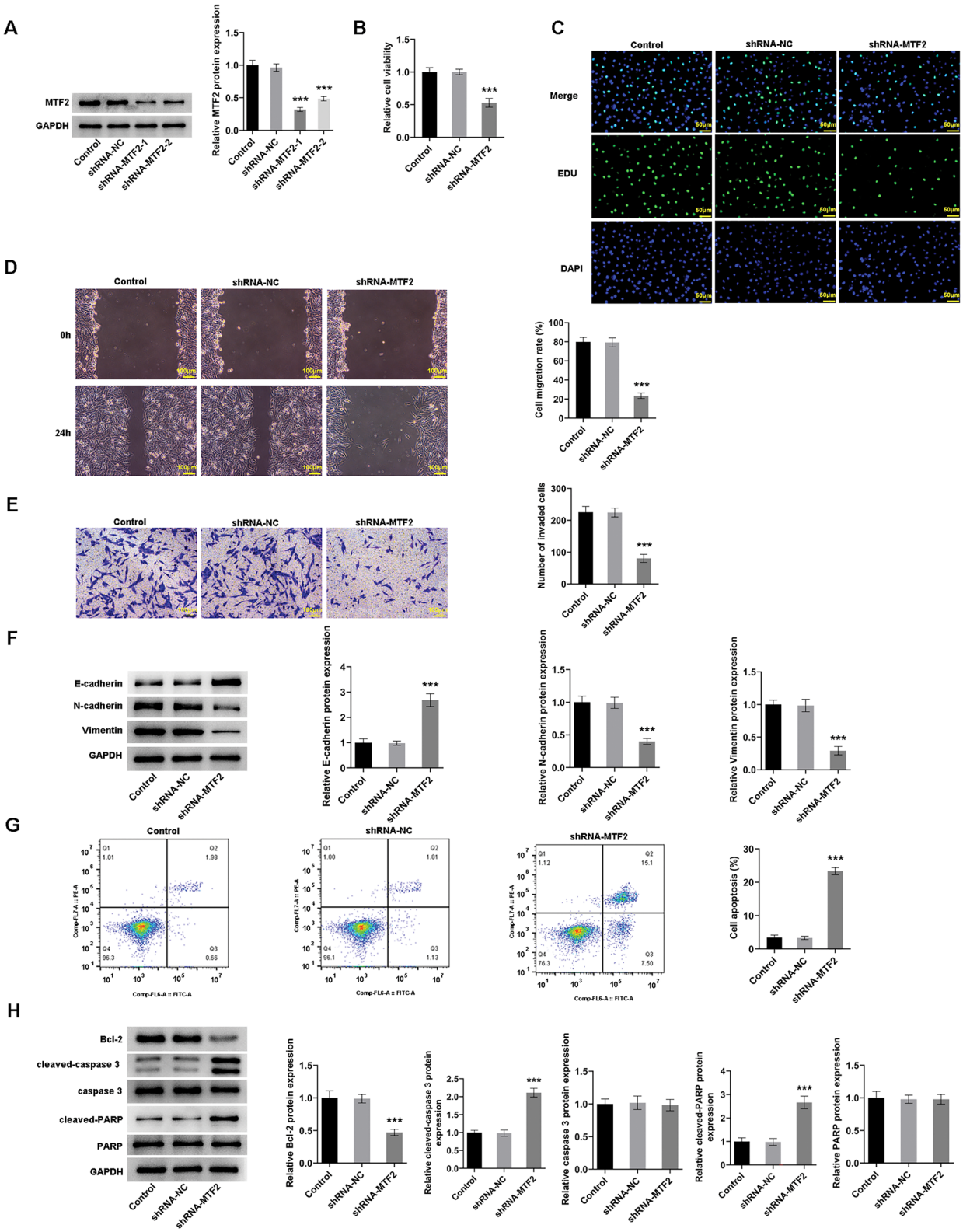

Fig. 2 MTF2 silence inhibits the aggressive biological events of MG-63 cells. **A** Examination of MTF2 expression by Western blot after transfection of shRNA-MTF2-1/2. **B** CCK-8 assay detected cell viability. **C** EDU staining detected cell proliferation. **D** Wound healing assay and **E** transwell assay measured cell migration and invasion. **F** Examination of EMT markers by Western blot. **G** Flow cytometry assay evaluated the cellular apoptotic rate. **H** Examination of apoptotic proteins by Western blot

while decreased N-cadherin and Vimentin expression in MG-63 cells (Fig. 2F). On the contrary, the apoptotic rate of MG-63 cells was significantly promoted by MTF2 silence (Fig. 2G), accompanied by reduced Bcl-2 expression and increased cleaved-caspase 3 and cleaved-PARP expression (Fig. 2H).

MTF2 interacts with EZH2 in MG-63 cells

To validate the interaction of MTF2 with EZH2 in osteosarcoma, EZH2 expression was examined. It turned out that EZH2 expression was increased in MG-63 cells compared with that in hFOB 1.19 cells (Fig. 3A). Specifically, EZH2 protein expression was found to be significantly reduced by interference with MTF2 (Fig. 3B). Results of Co-IP assay showed that MTF2 was enriched in EZH2 antibody and vice versa (Fig. 3C).

MTF2 deficiency up-regulates SFRP1 to inactivate Wnt signaling via EZH2

Interestingly, when MTF2 expression was silenced, Wnt and β-catenin expression were both reduced while Wnt inhibitor SFRP1 expression was increased (Fig. 4A). After EZH2 was overexpressed (Fig. 4B), Wnt, β-catenin expression were increased again and SFRP1 expression was decreased in MTF2-silencing MG-63 cells (Fig. 4C).

MTF2 silence mediates EZH2/SFRP1/Wnt signaling to function as a tumor suppressor in MG-63 cells

To explore the role of EZH2/SFRP1/Wnt signaling in the downstream mechanism of MTF2, EZH2 was overexpressed and SFRP1 antagonist WAY-316606 was used. As expected, the inhibited MG-63 cell viability due to MTF2 knockdown was partly increased again by EZH2 up-regulation or WAY-316606 treatment (Fig. 5A). As shown in Fig. 5B, MTF2 knockdown greatly inhibited the proliferation of MG-63 cells, which was then promoted again after EZH2 was overexpressed or following the pre-treatment with WAY-316606. Moreover, the inhibited cell migration and invasion due to MTF2 silence were both promoted again by EZH2 up-regulation or WAY-316606 pre-treatment (Fig. 5C-D). Consistently, EZH2 elevation or WAY-316606 treatment reduced E-cadherin expression and increased N-cadherin and Vimentin expression in MG-63 cells transfected with shRNA-MTF2 (Fig. 5E). In addition, EZH2 up-regulation or WAY-316606 pre-treatment significantly decreased the apoptotic level of MG-63 cells which was then facilitated by down-regulation of MTF2, accompanied by increased Bcl-2 expression and decreased cleaved-caspase 3 and cleaved-PARP expression (Fig. 5F-G).

Discussion

Osteosarcomas represents a primary mesenchymal tumor, the malignancy of which may involve a high degree of genetic heterogeneity (23).With the deep investigation into gene alternations, a growing body of research has identified various molecular markers for osteosarcoma such as PTHR1, CDK9 and CDC42EP3, and deepened the understandings of the etiology of osteosarcoma from the genetic and molecular perspectives

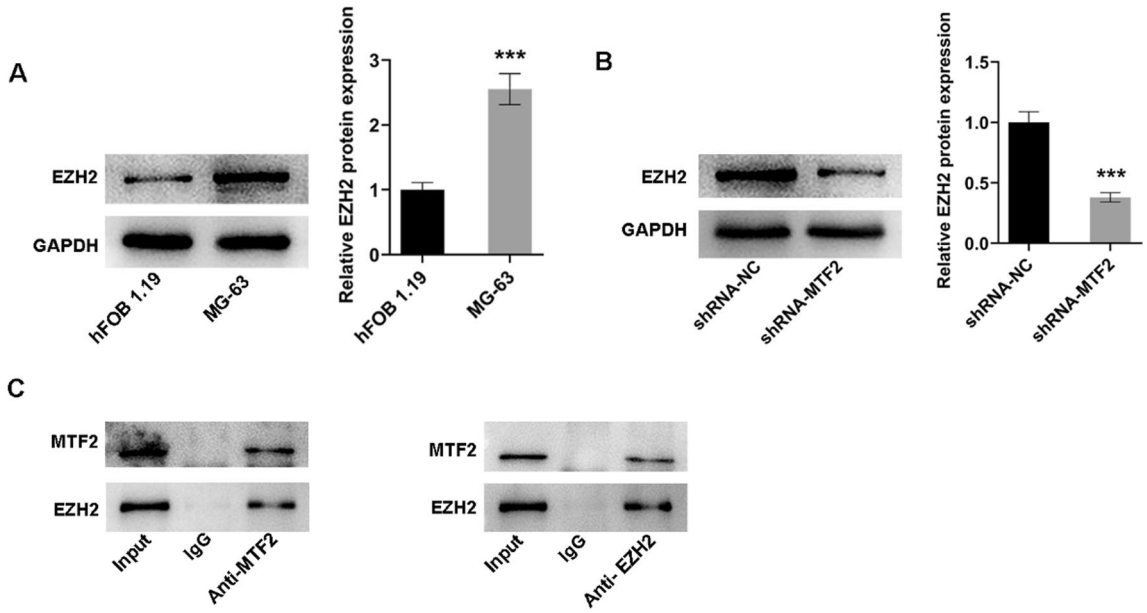

Fig. 3 MTF2 interacts with EZH2 in MG-63 cells. **A** Examination of EZH2 expression by Western blot in osteosarcoma cells. **B** Examination of EZH2 expression by Western blot after transfection of shRNA-MTF2. **C** Co-IP assay confirmed MTF2-EZH2 interaction

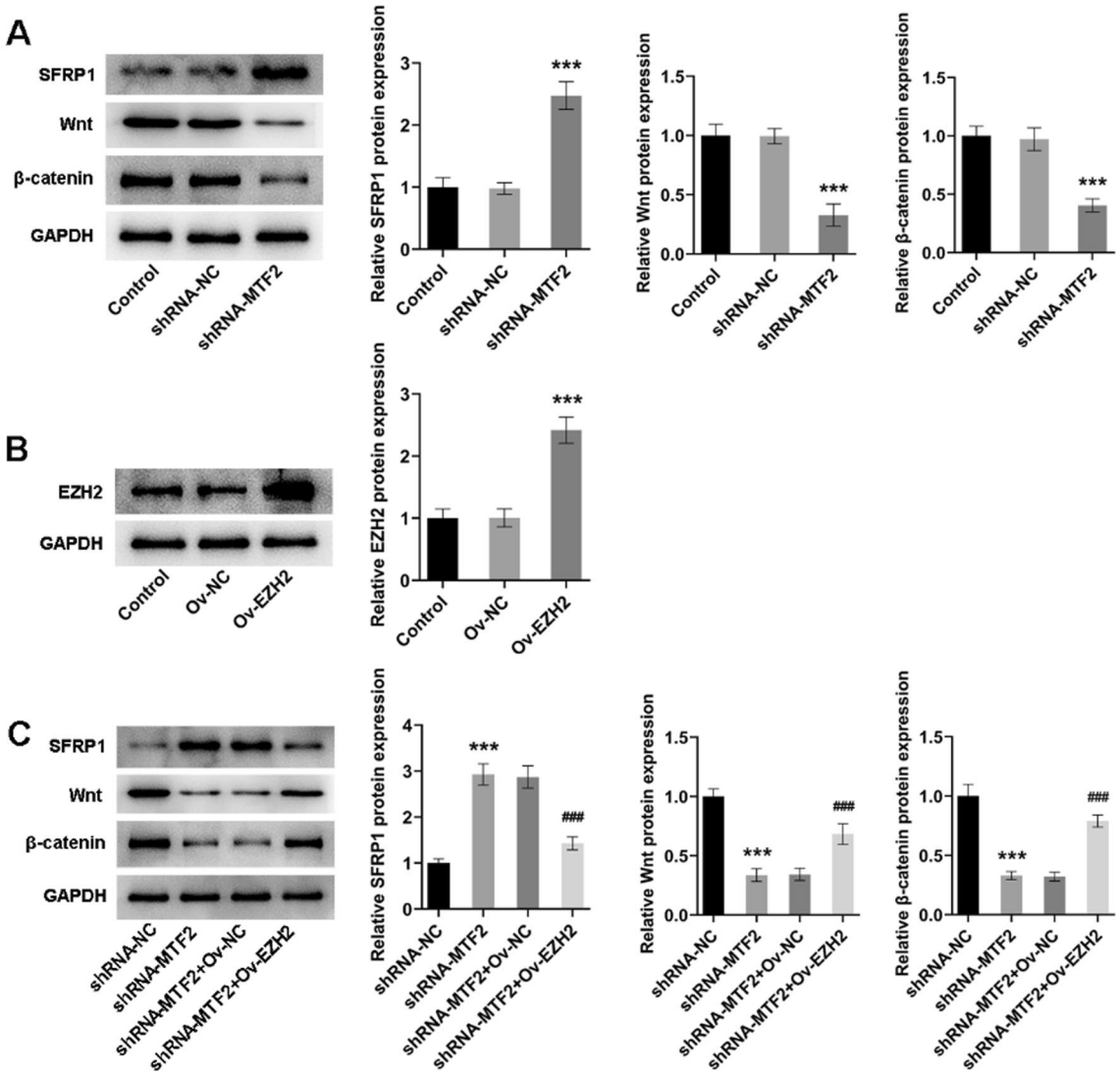

Fig. 4 MTF2 deficiency up-regulates SFRP1 to inactivate Wnt signaling via EZH2. **A** Examination of SFRP1/Wnt signaling-associated proteins by Western blot. **B** Examination of EZH2 expression by Western blot after transfection of Ov-EZH2. **C** Examination of SFRP1/Wnt signaling-associated proteins by Western blot

so far (24–26). Here we firstly presented the oncogenic role of MTF2 in osteosarcoma through accelerating the aggressive phenotypes of cells such as proliferation, migration, invasion and decreasing the cellular apoptotic level. In terms of the underlying action mechanism, MTF2 was assumed to interact with EZH2 to down-regulate SFRP1 and activate Wnt/β-catenin signaling.

MTF2 has been reported to play a significant role in the initiation and progression of cancers and act as an oncogene or tumor suppressor gene in a tissue-dependent manner (10, 11). Consistently, by GEO database, MTF2 expression was found to be increased in osteosarcoma tissues in our present work, which was consistent with the transcription co-factor-related gene signature and risk score model constructed by Jin et al. (12). The elevation of MTF2 was also observed in osteosarcoma cells, especially in MG-63 cells. As proposed by Wu et al., MTF2 can drive hepatocellular carcinoma cell proliferation (27). In this study, the viability and proliferation of osteosarcoma cells were both inhibited when MTF2 expression was silenced. Metastasis is dominantly characterized by uncontrolled proliferation of tumor cells and is commonly considered as a hallmark of human malignancies (28). Besides, as an essential event in the metastatic cascade, epithelial-mesenchymal transition (EMT), which is characterized by the loss of apical-basal

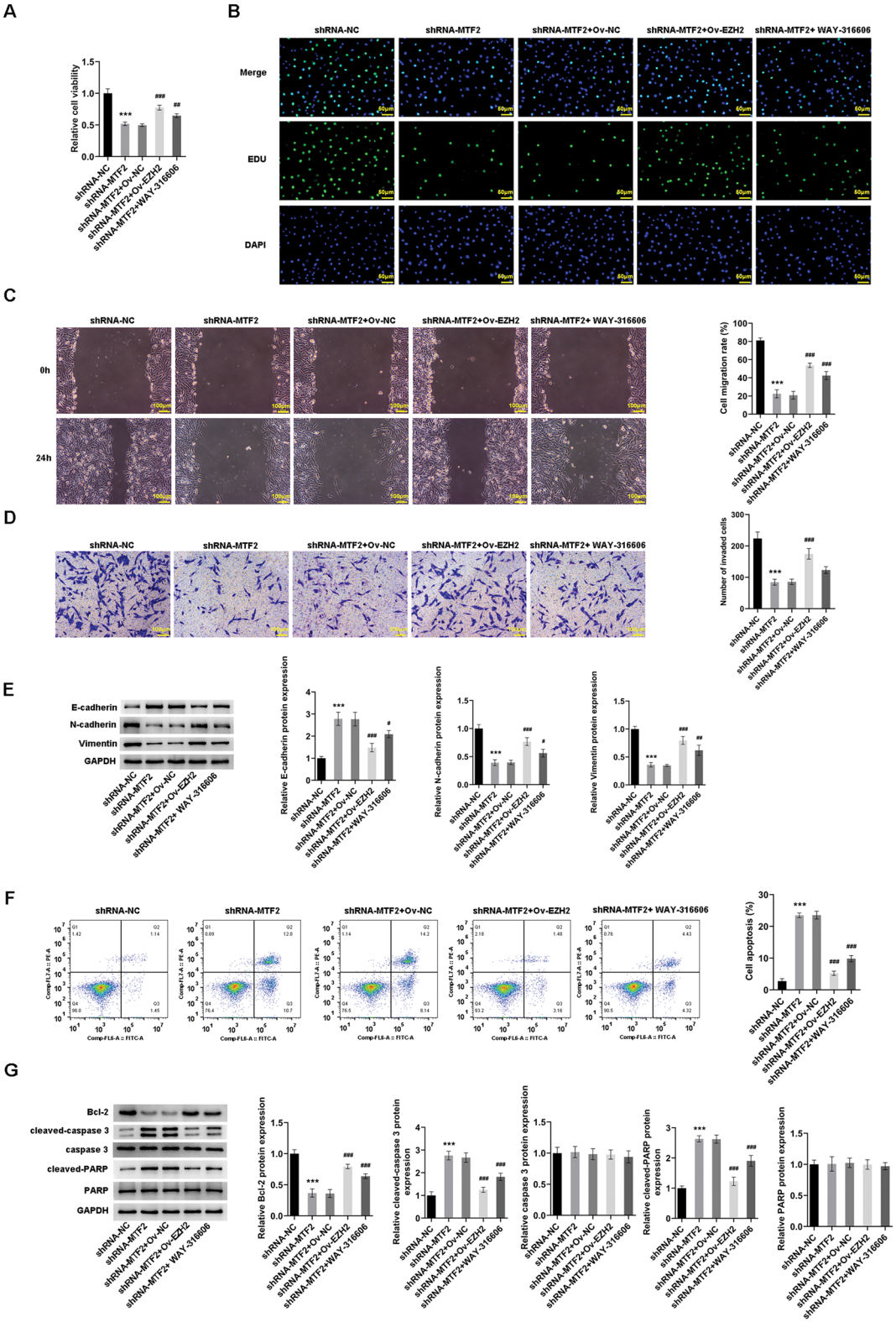

Fig. 5 MTF2 silence mediates EZH2/SFRP1/Wnt signaling to function as a tumor suppressor in MG-63 cells. **A** CCK-8 assay detected cell viability. **B** EDU staining detected cellular proliferation. **C** Wound healing assay and **D** transwell assay measured cell migration and invasion. **E** Examination of EMT markers by Western blot. **F** Flow cytometry assay evaluated the cellular apoptotic rate. **G** Examination of apoptotic proteins by Western blot

polarity and the looseness of cell–cell junctions to take on mesenchymal cell morphologies, is a program cell remodeling whereby the metastatic properties can be endowed to cancer cells by enhancing mobility, invasion, and resistance (29). E-cadherin is a transmembrane glycoprotein which connects epithelial cells together at adherens junctions (30). N-cadherin and Vimentin are mesenchymal markers responsible for the disassembly of adherens junctions (31, 32). Numerous reports have attested that MTF2 drives the migration, invasion and EMT of hepatocellular carcinoma and retinoblastoma cells (27, 33). Through investigation, in MG-63 cells, interference with MTF2 also inhibited the migration and invasion. Additionally, MTF2 knockdown up-regulated E-cadherin expression and down-regulated N-cadherin and Vimentin expression, suggesting the suppressive role of MTF2 knockdown in the transition of epithelial cells into a mesenchymal state in osteosarcoma cells. Further experimental results oppositely elaborated that MTF2 silence possessed the pro-apoptotic property in MG-63 cells, evidenced by reduced anti-apoptotic Bcl-2 expression and increased pro-apoptotic cleaved-caspase 3 and cleaved-PARP expression.

EZH2, the enzymatic subunit of PRC2 consisting of MTF2, has been reported to be reduced after MTF2 was knocked out (13). In this paper, the relationship between the two was first predicted by FpClass database. To verify this finding, Co-IP assay was performed and it was discovered that MTF2 and EZH2 interacted with each other in osteosarcoma cells. EZH2 is abnormally expressed and has shown therapeutic potential in various tumors through modulating a variety of cellular biological events, as a pleiotropic molecule (34). Emerging literatures have highlighted that EZH2 expression is highly expressed in osteosarcoma cells and promotes the malignant progression of osteosarcoma (14–16). In our current study, EZH2 was highly expressed in osteosarcoma cells. Moreover, it turned out that EZH2 expression was reduced by MTF2 silence, suggesting the positive correlation of MTF2 with EZH2. Further rescue experiments corroborated that EZH2 upregulation partly countervailed the influences of MTF2 silencing on the proliferation, metastasis and apoptosis of MG-63 cells in vitro.

SFRP1, a member of secretory glycoprotein SFRP family that is structurally related to Frizzled proteins, may directly bind to Wnt ligands or Frizzled receptors to act as an extracellular inhibitor involved in Wnt signaling (35). More interestingly, EZH2 can down-regulate target gene expression through catalyzing histone H3 lysine 27 (H3K27) trimethylation. Ample proof has substantiated that EZH2 directly targets SFRP1 and reduces SFRP1 expression which in turn activates Wnt/β-catenin signaling (18–20). The classical Wnt signaling is a conserved pathway that has been extensively implicated in embryonic development, tissue homeostasis, carcinogenesis and tumor metastasis relying on the function of the transcriptional coactivator β-catenin, and osteosarcoma is also included (36–38). Accumulative reports have also unmasked that SFRP1 inhibits the process of osteosarcoma mainly through inactivating the classical oncogenic Wnt/β-catenin signaling (21, 22). In addition, during erythropoiesis, MTF2 is deemed as a crucial epigenetic regulator of Wnt signaling (39). Our results reflected that MTF2 knockdown increased SFRP1 expression and decreased Wnt3a and β-catenin expression, which were reversed when EZH2 was up-regulated. As expected, following pretreatment with SFRP1 antagonist WAY-316606, the impacts of MTF2 silence on the proliferation, metastasis and apoptosis of MG-63 cells were also partly counteracted.

Conclusions

Conclusively, our study revealed a novel role of MTF2 in promoting the initiation and development of osteosarcoma via mediating EZH2/SFRP1/Wnt signaling. This finding might establish MTF2 and EZH2 as effective biomarkers for osteosarcoma and lay a solid theoretical and practical foundation for the future application of MTF2-targeted therapy in anti-osteosarcoma metastasis. There are certain limitations of the present study. First, the anti-tumor role of MTF2 in osteosarcoma has not been further proved in vivo and further animal studies will be done. Second, MTF2 is rarely studied in human malignancies and whether other signaling pathways are involved in MTF2 networks is worthy of being studied in the future.

Abbreviations

MTF2	Metal response element binding transcriptional factor 2
CCK-8	Cell Counting Kit-8
EDU	5-Ethynyl-2′-deoxyuridine
EMT	Epithelial mesenchymal transition
EZH2	Enhancer of zeste homolog 2
SFRP1	Secreted frizzled-related protein 1
Co-IP	Co-immunoprecipitation
PCL2	Polycomb-like 2
PRC2	Polycomb repressive complex 2
TCGA	The cancer genome atlas
ATCC	American type culture collection
DMEM	Dulbecco's modified eagle medium
EMEM	Eagle's minimum essential medium
FBS	Fetal bovine serum
shRNA	Short hairpin RNA
HRP	Horseradish peroxidase
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
RIPA	Radio immuno precipitation assay
BCA	Bicinchoninic acid
PVDF	Polyvinylidene fluoride
Bcl-2	B cell lymphoma-2
PARP	Poly-ADP-ribose polymerases
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
ANOVA	Analysis of variance

Author contributions
XH wrote the manuscript. XH and YL performed the experiments and analyzed the data. HS prepared the figures. TZ edited the manuscript. TL conceived the study. All authors reviewed the manuscript.

Funding
The authors raised funds themselves to perform this study.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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