Am J Transl Res 2025;17(2):1495-1509
www.ajtr.org /ISSN:1943-8141/AJTR0162942

Original Article

CCCTC-binding factor regulates splicing factor proline and glutamine-rich to promote malignant growth of osteosarcoma

Dapeng Li[1*], Yang Yang[1*], Zhengyu Yin[1], Lianghao Mao[2], Yiming Zhang[1], Pan Jiang[3], Tianxiang Zhu[4,5], Tongchuan He[6], Xinyu Zhong[1], Qiping Zheng[4,5,6], Wenchao Zhang[7]

[1]Spine Surgery, Affiliated Hospital of Jiangsu University, Zhenjiang 212001, Jiangsu, China; [2]Proteomics and Cancer Cell Signaling Group, German Cancer Research Center (DKFZ), Heidelberg, 69120, Germany; [3]Spine Surgery, Beijing Jishuitan Hospital Guizhou Hospital, Guiyang 550002, Guizhou, China; [4]Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, Jiangsu, China; [5]Shenzhen Walgenron Bio-Pharm Co. Ltd., Shenzhen 518118, Guangdong, China; [6]The Molecular Oncology Laboratory; Department of Orthopaedic Surgery and Rehabilitation Medicine, The University of Chicago Medical Center, Chicago, IL 60637, USA; [7]Affiliated Jintan Hospital of Jiangsu University, Changzhou 213200, Jiangsu, China. [*]Equal contributors and co-first authors.

Received December 26, 2024; Accepted February 6, 2025; Epub February 25, 2025; Published February 28, 2025

Abstract: Objectives: CCCTC-binding factor (CTCF) is a candidate tumor regulatory gene that encodes multifunctional transcription factors. While its role in various cancers has been studied, its function and mechanism in osteosarcoma were uncertain. Previous studies have identified splicing factor proline and glutamine-rich (SFPQ) as an oncogene in osteosarcoma. Bioinformatic analysis suggested that CTCF may regulate SFPQ transcriptionally. This study aimed to elucidate the role of CTCF in osteosarcoma and explore its possible regulatory relationship with SFPQ. Methods: Potential transcription factors of SFPQ were identified using an online transcription factor analysis database. The expression levels of CTCF in osteosarcoma cells were assessed using quantitative real-time PCR (qRT-PCR) and western blotting (WB). The effect of CTCF and SFPQ on osteosarcoma cell behavior was evaluated through cell function assays, dual-luciferase reporter assays, and rescue experiments. Results: Database analyses (hTFtarget and GEPIA2) indicated a moderate correlation between CTCF and SFPQ. qRT-PCR and WB results confirmed significant CTCF expression in osteosarcoma cells. Overexpression of CTCF enhanced cell proliferation, migration, and invasion. Furthermore, CTCF was found to bind to the promoter region of SFPQ, leading to its upregulation. Rescue experiments demonstrated that SFPQ knockdown attenuated the oncogenic effects of CTCF overexpression. Conclusions: CTCF functions as an oncogene in osteosarcoma by positively regulating SFPQ expression, thereby promoting the malignant properties of osteosarcoma cells. These findings suggest that targeting the CTCF-SFPQ axis may be a therapeutic strategy for osteosarcoma.

Keywords: Osteosarcoma, CTCF, SFPQ, transcription factor, biological behavior

Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor, followed by Ewing's sarcoma and chondrosarcoma. It primarily arises in the metaphysis of long bones [1]. The incidence of OS peaks at two distinct age periods: the first during adolescence, the primary age group affected, and the second in individuals over 65 years old [2, 3]. OS is highly aggressive, rapidly progressive, and prone to early metastasis, making it a leading cause of cancer-related mortality among teenagers [4]. The gold standard for diagnosing osteosarcoma remains tissue biopsy [5]. Common serological markers, such as alkaline phosphatase and lactate dehydrogenase, are frequently used; however, their clinical utility is limited due to poor sensitivity and specificity [6]. Advances in medical technology have significantly improved the 5-year survival rate for non-metastatic OS, increasing it from 20% to 70% [2].

https://doi.org/10.62347/STQK5435

However, the prognosis for patients with metastatic, recurrent, or chemotherapy-resistant OS remains poor, with the 5-year survival rate still critically low. Given these challenges, understanding the biological mechanisms underlying osteosarcoma progression is essential. Identifying effective therapeutic targets and biomarkers for early detection could significantly improve clinical outcome and allow targeted treatment.

Splicing factor proline and glutamine rich (SFPQ) is primarily localized in the nucleus and plays a crucial role in various nuclear functions, including RNA transport, DNA repair, splicing, and transcriptional regulation [7]. Previous studies have shown that SFPQ's structural domains can bind to the promoters of multiple genes and regulate transcription, demonstrating the ability to interact with both DNA and RNA [8, 9]. Proteomic research has identified SFPQ as being highly abundant in sarcoma cells, suggesting a role in tumorigenesis when compared to non-malignant mesenchymal cells [10]. Our recent findings indicate that SFPQ is highly expressed in osteosarcoma cells and tissues. Functional experiments revealed that SFPQ knockdown significantly reduced osteosarcoma cell migration, invasion, and proliferation, whereas SFPQ overexpression enhanced these oncogenic properties, reinforcing its role in osteosarcoma progression [11]. However, the precise mechanisms underlying SFPQ regulation in osteosarcoma, particularly its upstream regulatory factors, remain to be fully elucidated.

The CCCTC-binding factor (CTCF) plays a diverse range of regulatory roles in physiologic processes, including genome organization, enhancer blocking, DNA methylation, imprinting, and transcriptional control. It is a highly conserved transcription factor characterized by 11 zinc fingers [12]. The Encyclopedia of DNA Elements (ENCODE) project has identified widely distributed CTCF binding sites across different cell types and revealed distinct differences in CTCF binding patterns between normal and tumor cells [13]. Additionally, CTCF is a key component of chromatin's three-dimensional structure, contributing to the formation of chromatin loops and topologically associated domains (TADs) [12, 14, 15]. By regulating chromosome architecture, CTCF plays a crucial role in gene expression and protein translation, significantly influencing tumor development and progression [16].

Studies have shown that mutations in CTCF can disrupt its ability to bind DNA, impairing its genomic recognition and leading to malignant tumor development [17]. Indeed, CTCF mutations have been identified in various cancers, including liver, breast, lung, and prostate cancer, where they influence the growth and proliferation of malignant cells by altering transcriptional regulation [18-21]. Interestingly, high levels of CTCF expression have been detected in the exosomes of drug-resistant osteosarcoma cells. Research suggests that CTCF can activate autophagy-dependent pathways, thereby enhancing cisplatin resistance in osteosarcoma cells [22]. However, the specific role of CTCF in osteosarcoma progression remains largely unexplored. In this study, we aim to investigate the transcriptional regulation of SFPQ by CTCF in osteosarcoma through *in vitro* experiments. Additionally, we seek to determine how CTCF-mediated regulation of SFPQ influences osteosarcoma cell invasion, migration, and proliferation.

Materials and methods

Relevance analysis

To analyze the relevance of CTCF and SFPQ, we used the hTFtarget (Database of Human Transcription Factor Targets). By accessing the database (https://guolab.wchscu.cn), we entered "SFPQ" in the "Target" search page and selected "Details" in the CTCF column. This provided the binding site of SFPQ within the CTCF sequence. For correlation analysis, we used the GEPIA (Gene Expression Profiling Interactive Analysis) database (http://gepia.cancer-pku.cn). By selecting the "Correlation Analysis" tool, we input "CTCF" and "SFPQ", then chose "SARC tumor", obtaining the correlation analysis between CTCF and SFPQ.

Additionally, in the GEPIA2 database (http://gepia2.cancer-pku.cn), we searched for "CTCF" to assess its pan-cancer expression profile.

Cells and cell culture

The osteosarcoma cell lines used in this experiment included HOS, 143B, U2OS, and

Table 1. Primers used for qRT-PCR analysis

Gene	Sequence (5' to 3')
CTCF-F	5'-CAGTGGAGAATTGGTTCGGCA-3'
CTCF-R	5'-CTGGCGTAATCFCACATGGA-3'
SFPQ-F	5'-TCCACACCAACAGCAGCAACAG-3'
SFPQ-R	5'-GCAACGACGGGCTTGGAAGAG-3'
GAPDH-F	5'-CAGGAGGCATTGCTGATGAT-3'
GAPDH-R	5'-GAAGGCTGGGGCTCATTT-3'

Table 2. All siRNAs used for cell transfection

siRNA	Sequence (5' to 3')
NC sense	5'-UUCUCCGAACGUGGUCACGUTT-3'
NC antisense	5'-ACGUGACACGUUCGGAGAATT-3'
si-1 sense	5'-GGUCUGCUAUCAGAGGUUATT-3'
si-1 antisense	5'-UAACCUCUGAUAGCAGACCTT-3'
si-2 sense	5'-GUGCAAUUGAGAACAUUAUTT-3'
si-2 antisense	5'-AUAAUGUUCUCAAUUGCACTT-3'
si-3 sense	5'-GGUGGAGACACUAGAACAATT-3'
si-3 antisense	5'-UUGUUCUAGUGUCUCCACCTT-3'

MG63, along with normal human osteoblasts (hFOB1.19). All cell lines were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences.

Cell culture media were prepared using fetal bovine serum (FBS) and complete medium, with specific conditions for each cell line. For hFOB1.19 (human osteoblast cell line), we cultured in D-MEM/F-12 medium supplemented with 12% FBS. For HOS and MG63 (osteosarcoma cell lines) , we cultured in DMEM medium supplemented with 10% FBS. For U2OS cell line, we cultured in McCoy's 5A medium supplemented with 10% FBS. For143B cell line, we cultured in RPMI 1640 medium supplemented with 10% FBS. All cell lines were validated using short tandem repeat (STR) profiling and tested negative for mycoplasma contamination. hFOB1.19 osteoblasts were maintained at 33.5°C with 5% CO_2, while osteosarcoma cells were kept at 37°C with 5% CO_2.

Quantitative real-time PCR

Total RNA was extracted from cells cultured in Petri dishes using RNA-easy reagent (Vazyme, China). cDNA synthesis was performed using the PrimeScript™ RT Master Mix (TaKaRa, Japan), following the manufacturer's instructions.

Quantitative real-time PCR (qRT-PCR) was conducted to analyze target gene expression levels using the UltraSYBR Mixture (CWBIO, China). The primer sequences used in this study are listed in **Table 1** below.

Protein extraction and western blot analysis

Total cellular protein was extracted using RIPA buffer (Millipore, USA) supplemented with protease inhibitors. Protein samples were separated via 10% SDS-PAGE electrophoresis and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with TBST containing 5% skim milk powder for 1 hour at room temperature, followed by overnight incubation at 4°C with primary antibodies against GAPDH (Danvers, MA, USA) or CTCF (Abcam, USA).

The next day, the membrane was incubated with a goat anti-rabbit secondary antibody for 1 hour, then washed three times with TBST. Protein bands were visualized using a bioimaging system (Bio-Rad, USA) with an enzyme-linked chemiluminescence (ECL) detection kit.

Cell transfection

Cells were seeded into 6-well plates and cultured until reaching 30-40% confluency the following day. Transfection was performed using three siRNAs targeting CTCF, a negative control (NC) siRNA, a CTCF overexpression plasmid, or a pcDNA3.1 vector as a negative control (RiboBio, China). The transfection mixture was prepared following the Lipofectamine 3000 transfection protocol (Invitrogen, USA). The transfection mixture was thoroughly mixed with the culture medium, and cells were incubated for 6 hours before replacing the medium. After an additional 48-hour incubation, cells were collected for further experiments.

The knockdown efficiency was confirmed by qRT-PCR, which indicated that si-2 (si-CTCF) exhibited the most significant knockdown effect. Therefore, all subsequent experiments were conducted using si-2. All siRNA sequences used in this study are listed in **Table 2**.

Cell scratching assay

Before the experiment, using a straightedge and a marker, horizontal lines were drawn on

the back of the 6-well plate in equal intervals, with lines 0.8 cm apart across the wells, ensuring at least five lines per well. Cells were transfected according to the standard protocol and continuously cultured for 48 hours. When the cell growth density reached approximately 95%, a cell scratching experiment was performed. Using a 100 microliter pipette tip and a straightedge as a guide, horizontal lines were scratched at the bottom of the wells, ensuring the pipette tip was held perpendicular to the well bottom and not tilted. After 0, 24, and 48 hours of incubation, the cells were examined under a 10× microscope, and photographs were taken to document the findings. The images were saved for analysis.

Cell plate colony assay

Cells in the logarithmic growth phase that had been transfected were collected and counted. A total of 1,000 cells per well were seeded into 6-well plates according to different experimental groups. The cells were continuously cultured for 12 to 14 days until individual scattered cells formed visible colonies.

Once colonies had formed, the cells were washed with PBS and fixed with 600 μL of 4% paraformaldehyde for 30 minutes. After removing the paraformaldehyde, 600 μL of 0.1% crystal violet solution was added for 30 to 60 minutes to stain the colonies. Following three washes with PBS, the plates were air-dried at room temperature, and the results were photographed for documentation.

Cell counting Kit-8 (CCK-8) assay

Experimental and control group cells were set up according to the cell transfection method. In a 96-well plate, 100 μL of cell suspension was added to each well to ensure each well contained 5,000 cells. Each experimental and control group was set up in 4 columns, with 6 sub-wells in each column. After 24 hours, the CCK-8 reagent and cell culture medium were mixed at a 1:9 ratio to prepare the CCK-8 working solution. 100 μL of this working solution was added to the first column of wells to be tested. The cells were then incubated in a cell culture incubator for 1 hour, and the OD values of each well in the first column were recorded using a microplate reader (with the wavelength set to 450 nm). The following day, OD values were measured for each well in the second column of each group. This process was repeated for 4 consecutive days, recording OD values daily.

Transwell migration and invasion assay

Cells from different treatment groups were transfected and cultured until reaching the logarithmic growth phase. The cell density was adjusted to 80,000 cells/mL. After carefully inserting the chambers into the wells of a 24-well cell culture plate, 600 μL of cell culture medium was added to each well, and 200 μL of cell suspension was added to each chamber corresponding to the experimental groups. The cells were cultured for an additional 24 hours. After 24 hours, the Transwell chambers were removed, fixed in 4% paraformaldehyde, stained with crystal violet, and dried at room temperature. The cells were observed under a 10× microscope, and appropriate fields of view were selected for photography to document the results. For the invasion experiments, a matrix gel was pre-arranged in the upper layer of the chambers. To do this, 70 μL of matrix gel dilution was added to each chamber, spread evenly over the bottom, and placed in an incubator for 4 hours to allow the gel to solidify. Once the matrix gel was solidified, the procedures for the Transwell migration experiments were followed. The cell inoculation density for the invasion experiment was 2 × 10^5 cells/mL, and the cell culture time was extended to 48 hours. The cells were then fixed, stained, and photographed using the same method as described above.

The 5-ethynyl-20-deoxyuridine (EDU assay)

Cells that had been transfected and cultured to the logarithmic growth phase were taken for cell counting, and 1×10^5 cells per well were inoculated into the wells of a 96-well plate and cultured for 24 hours to reach the logarithmic growth phase. The cells were then cultured for another 24 hours using the EdU Cell Proliferation Detection Kit (Rui Bo, China) according to the manufacturer's instructions. The cells were sequentially subjected to paraformaldehyde fixation, glycine incubation, and TritonX-100 shaker incubation. Each well was filled with 100 μl of 1× Apollo staining reaction solution and allowed to sit at room temperature for 30 minutes for decolorization. This

was followed by decolorization and washing with 100 μl of the osmotic agent shaker 2-3 times, each for 10 minutes. Following the addition of 100 μl of 1× Hoechst 33342 reaction solution to each well, the plates were allowed to sit at room temperature on a shaker for 30 minutes in the dark. Afterward, the wells were washed with 100 μl of PBS on a shaker for decolorization. Finally, 100 μl of PBS was added to each well for storage. The results were observed under a fluorescence inverted microscope within 3 days and photographed.

Dual-luciferase reporter assay

The dual-luciferase plasmid was constructed by Guangzhou RuiBo Company. The reporter gene plasmid was incorporated with a 2000 bp nucleotide sequence upstream of SFPQ (SFPQ promoter sequence). 293T cells were cultured in 24-well plates at a density of 5×10^4 cells/well and left to incubate for 24 hours. Cells were then transfected with co-transfection mixtures, prepared according to the kit instructions (Promega, USA), and incubated for an additional 48 hours. Cell lysates were prepared using Cell Lysis Buffer, and the supernatant was collected for subsequent assays. A Luciferase Substrate solution was added to an assay tube, followed by the addition of the cell lysate supernatant. The mixture was thoroughly mixed and placed in a fluorescence detector to measure Firefly luciferase reporter gene activity. Subsequently, a Renilla substrate working solution was added to the same tube, and the tube was immediately placed in the fluorescence detector to measure Renilla luciferase reporter gene activity.

The luciferase activity of fireflies was normalized to that of Renilla for comparison. The activity ratios were calculated, and bar graphs were plotted and analyzed using GraphPad Prism 8.0 software.

Statistical analysis

The experimental data were processed using SPSS software for statistical analysis. Graphs of the experimental results were created and plotted using GraphPad Prism 8.0 software. The experimental data were expressed as the mean ± standard deviation. Statistical significance was determined with $P < 0.05$ considered significant.

Results

CTCF is a potential transcriptional regulator for SFPQ and is highly expressed in osteosarcoma cells

Using the hTFtarget website, we identified CTCF as a potential transcriptional regulator of SFPQ (**Figure 1A**). Further analysis using the GEPIA2 database validated this finding, establishing a moderate correlation between CTCF and SFPQ expression ($R = 0.54$). Additionally, pan-cancer expression level analysis revealed high expression of CTCF in sarcoma tissues (**Figure 1B**, **1C**).

According to the analysis of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) screen dataset from the Dependency Map portal (DepMap) database (https://depmap.org/portal/), the gene effect score of CTCF in osteosarcoma cells is significantly less than -1 (indicating that knockdown of CTCF inhibits osteosarcoma cell proliferation). This also showed that CTCF is highly expressed in all osteosarcoma cells (Supplementary Figure 1A, 1B).

RT-PCR and western blot (WB) analyses were conducted to determine the expression levels of CTCF in osteoblasts and osteosarcoma cells. The qRT-PCR results demonstrated that osteosarcoma cells exhibited higher CTCF mRNA expression levels compared to osteoblasts. Similarly, the WB data showed that the protein expression level of CTCF was consistent with the qRT-PCR results, with the differences being significant (**Figure 1D**, **1E**).

Knockdown of CTCF inhibits the proliferation, migration and invasion of osteosarcoma cells

WB and qRT-PCR data revealed that after transfection of HOS and 143B cells with si-CTCF, CTCF levels were significantly reduced in both cell lines (**Figure 2A**, **2B**). Plate colony formation experiments showed a significant reduction in the clonal colony formation of HOS and 143B cells, indicating that CTCF knockdown markedly inhibited cell proliferation (**Figure 2C**). According to the EdU and CCK-8 assays, cells from the knockdown group exhibited much lower OD values and fewer proliferating cells compared to the control group (**Figure 2D**, **2E**).

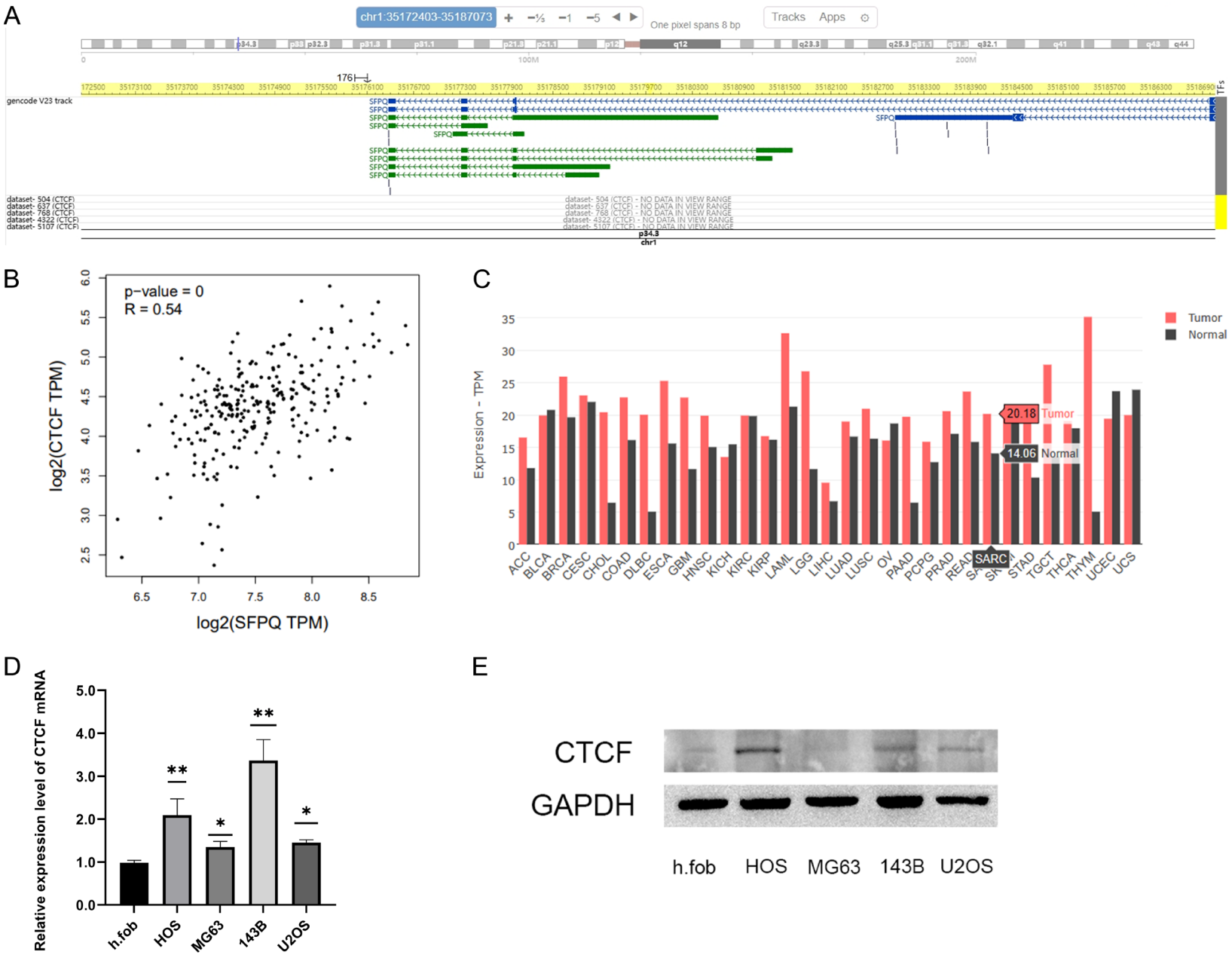

A
chr1:35172403-35187073
One pixel spans 8 bp
Tracks
Apps
gencode V23 track
SFPQ
B
p−value = 0
R = 0.54
log2(CTCF TPM)
log2(SFPQ TPM)
C
Expression - TPM
Tumor
Normal
20.18 Tumor
14.06 Normal
SARC
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS
D
Relative expression level of CTCF mRNA
h.fob
HOS
MG63
143B
U2OS
E
CTCF
GAPDH
h.fob
HOS
MG63
143B
U2OS

Figure 1. The CCCTC-binding factor (CTCF) is a potential transcription factor for splicing factor proline and glutamine-rich (SFPQ) and is highly expressed in osteosarcoma cells. The hTFtarget (Database of Human Transcription Factor Targets) website (https://guolab.wchscu.cn) indicates that CTCF may be a transcription factor for SFPQ (A). The GEPIA (Gene Expression Profiling Interactive Analysis) database (http://gepia.cancer-pku.cn) showed a moderate correlation between SFPQ and CTCF expression (B), and the GEPIA2 database (http://gepia2.cancer-pku.cn) revealed that CTCF is highly expressed in sarcoma tissues (C). Expression levels of CTCF mRNA and protein in osteosarcoma cells and osteoblasts are shown (D, E). The molecular weight of CTCF is 140 kDa, and GAPDH is 36 kDa. (Results are displayed as the mean ± SD; $^{**}P < 0.01$, $^{*}P < 0.05$; n = 3).

The cell scratch assays demonstrated that both cell types in the knockdown group migrated more slowly and at a significantly lower rate compared to the control group (**Figure 2F**). Results from Transwell migration and invasion assays showed that there were significantly fewer migrating and invading cells in the knockdown group, and both HOS and 143B cells exhibited dramatically reduced migration and invasion capacities (**Figure 2G**, **2H**).

Overexpression of CTCF enhances the proliferation, migration and invasion of osteosarcoma cells

qRT-PCR and WB results indicated that after transfection with the CTCF overexpression plasmid in HOS and 143B cells, CTCF levels in both cell lines were significantly increased (**Figure 3A**, **3B**). In the cell plate colony formation assays, the overexpression group exhibited significantly higher clonal colony formation in HOS and 143B cells compared to the control group, with a notable increase in cell proliferation ability (**Figure 3C**). CCK-8 and EdU assay results showed that the OD value and the number of cells in the proliferative state were significantly higher in the overexpression group compared to the control group (**Figure 3D**, **3E**). Cell scratch assays demonstrated that the migration speed of cells was increased, and the migration rate was significantly enhanced in the overexpression group (**Figure 3F**).

Transwell assays revealed that both the migration and invasion capacities of the cells were significantly higher when CTCF was overexpressed (**Figure 3G**, **3H**).

CTCF affects the biological behavior of osteosarcoma cells by regulating SFPQ

To verify whether a targeted regulatory relationship exists between CTCF and SFPQ, we performed a dual-luciferase reporter assay. The assay revealed that relative luciferase activity was enhanced when the CTCF overexpression plasmid was co-transfected with the reporter gene vector, compared to the negative control (**Figure 4A**). These findings suggest a specific regulatory connection between CTCF and SFPQ.

To elucidate the mechanism by which CTCF regulates SFPQ expression, qRT-PCR and WB were conducted in HOS cells. The results showed that SFPQ expression was markedly increased at both the mRNA and protein levels upon CTCF overexpression (**Figure 4B**, **4C**), indicating that CTCF directly targets and regulates SFPQ expression.

Rescue experiments were then performed in HOS cells. In cell plate colony formation assays, cells overexpressing CTCF displayed a significantly higher capacity to form colonies. However, when cells were transfected with si-SFPQ, this colony-forming ability decreased. Notably, co-transfection of the CTCF plasmid and si-SFPQ resulted in a weakened colony-forming capacity compared to cells with CTCF overexpression alone (**Figure 4D**).

Similarly, CCK-8 and EdU assays showed trends consistent with the colony formation assay results (**Figure 4E**, **4F**), indicating that the increased proliferative ability induced by CTCF overexpression was partially offset by SFPQ knockdown.

Furthermore, the cell scratch assay demonstrated that the enhanced migration of HOS cells due to CTCF overexpression could be partially reversed by SFPQ knockdown (**Figure 4G**). In the Transwell migration and invasion assays, fewer cells were observed in the co-transfection group (CTCF overexpression + si-SFPQ) compared to the CTCF overexpression group alone, suggesting that SFPQ knockdown partially counteracted the enhanced migration and invasion induced by CTCF (**Figure 4H**).

Collectively, these findings suggest that the enhancement of osteosarcoma cell prolifera-

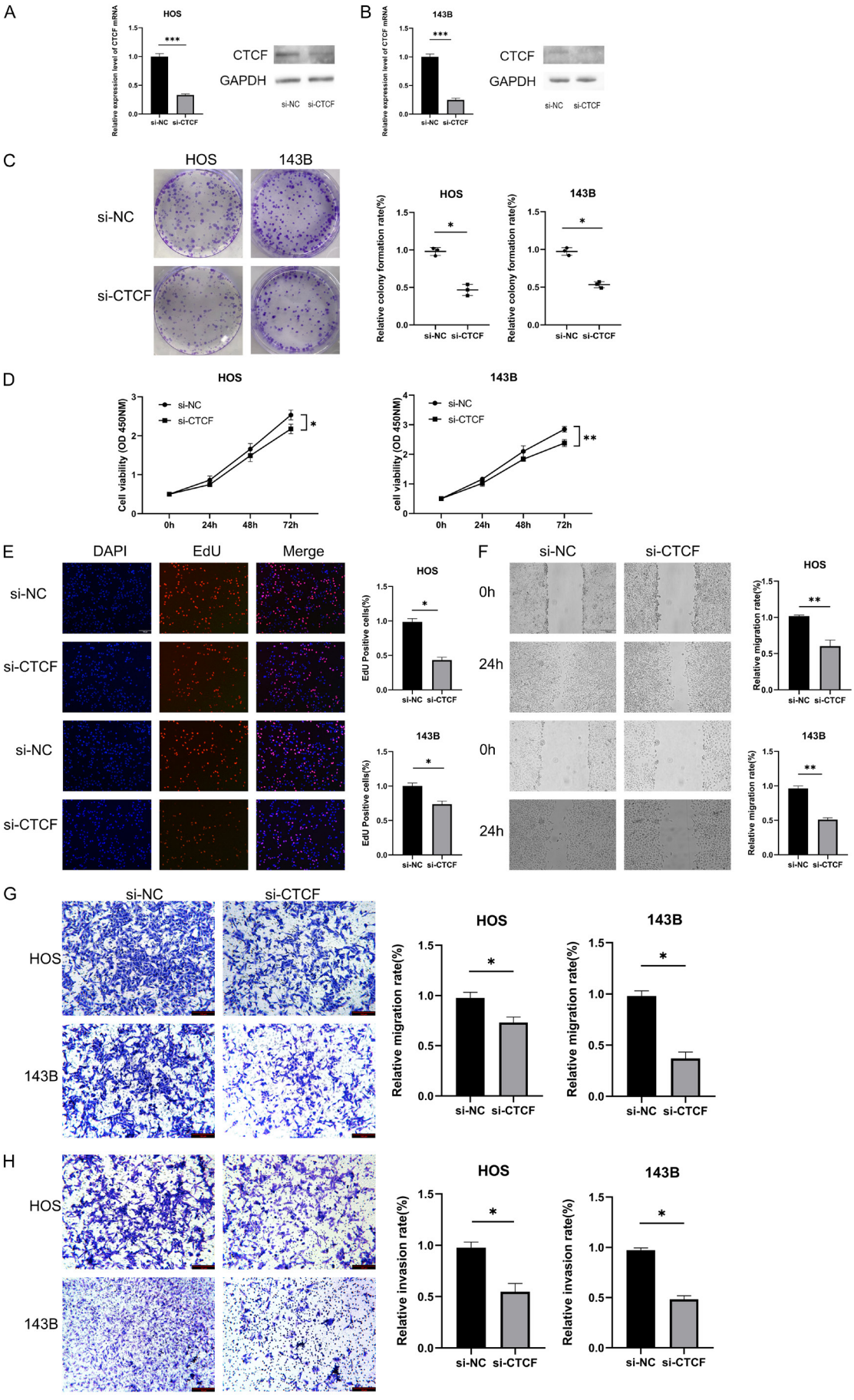

A
HOS
Relative expression level of CTCF mRNA
***
si-NC si-CTCF
CTCF
GAPDH
si-NC si-CTCF
B
143B
Relative expression level of CTCF mRNA
***
si-NC si-CTCF
CTCF
GAPDH
si-NC si-CTCF
C
HOS
143B
si-NC
si-CTCF
HOS
Relative colony formation rate(%)
*
si-NC si-CTCF
143B
Relative colony formation rate(%)
*
si-NC si-CTCF
D
HOS
Cell viability (OD 450NM)
si-NC
si-CTCF
*
0h 24h 48h 72h
143B
cell viability (OD 450NM)
si-NC
si-CTCF
**
0h 24h 48h 72h
E
DAPI
EdU
Merge
si-NC
si-CTCF
si-NC
si-CTCF
HOS
EdU Positive cells(%)
*
si-NC si-CTCF
143B
EdU Positive cells(%)
*
si-NC si-CTCF
F
si-NC
si-CTCF
0h
24h
0h
24h
HOS
Relative migration rate(%)
**
si-NC si-CTCF
143B
Relative migration rate(%)
**
si-NC si-CTCF
G
si-NC
si-CTCF
HOS
143B
HOS
Relative migration rate(%)
*
si-NC si-CTCF
143B
Relative migration rate(%)
*
si-NC si-CTCF
H
HOS
143B
HOS
Relative invasion rate(%)
*
si-NC si-CTCF
143B
Relative invasion rate(%)
*
si-NC si-CTCF

Figure 2. Knockdown of CTCF inhibits the proliferation, migration, and invasion of osteosarcoma cells. Detection of CTCF knockdown efficiency in HOS (A) and 143B (B) cells by quantitative real-time PCR (qRT-PCR) and western blot (WB). Effects of CTCF knockdown on the proliferative ability of osteosarcoma cells were assessed by cell plate cloning assay, Cell Counting Kit-8 (CCK-8) experiment, and 5-ethynyl-20-deoxyuridine (EdU) assay. Scale bar = 100 μm (C-E). Effects of CTCF knockdown on migration and invasion abilities of osteosarcoma cells were detected by cell scratch test (F), and Transwell migration and invasion assay (G, H). The scale bar in the cell scratch test is 100 μm, and the scale bar in Transwell migration and invasion assay is 50 or 100 μm. (Results are displayed as the mean ± SD, ***P < 0.001, **P < 0.01, *P < 0.05; n = 3).

tion, migration, and invasion induced by CTCF overexpression is partially abrogated by co-transfection with si-SFPQ. This supports the conclusion that CTCF promotes osteosarcoma cell proliferation, motility, and invasion through the regulation of SFPQ.

Discussion

Osteosarcoma is the most common and aggressive primary malignant tumor in adolescents. It primarily arises from mesenchymal cells and is characterized by the formation of bone-like tissue [23]. Osteosarcoma usually develops in the metaphysis of long bones, particularly in the proximal tibia and distal femur [24]. The pathogenesis of osteosarcoma is complex, with exposure to radiation and alkylating agents serving as significant risk factors [25, 26]. The tumor is marked by rapid growth, early metastasis, and a high degree of malignancy, making the selection of appropriate treatment options a major clinical challenge [23, 27].

Currently, the standard treatment for osteosarcoma involves surgical intervention, including amputation and limb-sparing surgery; however, the exact extent of resection remains controversial [28]. Metastatic, recurrent, and drug-resistant osteosarcomas respond poorly to treatment, resulting in a 5-year survival rate of only 20% [29]. Aberrant gene expression and gene mutations are closely linked to the onset and spread of osteosarcoma [30, 31]. Since osteosarcoma does not present with precancerous lesions or carcinoma in situ, it is often diagnosed at an advanced stage, leading to a very poor prognosis [32]. Therefore, investigating the molecular mechanisms underlying osteosarcoma onset and progression, as well as identifying biomarkers for early detection and diagnosis, is of paramount importance [33].

In addition to its essential role in stem cell development, SFPQ is crucial for cancer development and progression [34-37]. SFPQ plays a complex, tissue-specific regulatory role in malignant tumors. For instance, in breast and prostate cancers, SFPQ drives oncogenic progression by post-transcriptionally regulating key genes [38, 39]. Conversely, in lung and kidney cancers, SFPQ functions as a transcriptional repressor by suppressing proto-oncogene expression [40, 41]. A related investigation found that SFPQ is nearly undetectable in non-malignant cells but is substantially expressed in various malignant tumor cells, with particularly high levels in sarcoma cells-consistent with our observations [10]. These results suggest that in osteosarcoma, SFPQ acts as an oncogene. However, the specific mechanisms by which SFPQ influences osteosarcoma cells remain to be determined.

It is well-established that abnormal expression of transcription factors significantly affects cellular metabolism and signaling in cancer, and approximately 294 cancer-related transcription factors have been identified and cataloged in various databases [42]. Therefore, identifying and targeting tumor-specific transcription factors and their regulators may open new avenues for cancer treatment [43]. In this study, we investigated the molecular mechanism by which SFPQ functions as an oncogene in osteosarcoma. Candidate transcription factors targeting SFPQ were screened using multiple transcription factor databases, leading to the identification of CTCF as a candidate regulator for subsequent experiments.

There is growing evidence that CTCF is critical for nuclear spatial organization and is involved in various genetic and epigenetic mechanisms through transcriptional regulation [44]. CTCF regulates a wide array of genes associated with tumor development, particularly those involved in growth, proliferation, differentiation, and apoptosis [45-47]. Moreover, CTCF function is influenced by interactions with protein chaperones and by post-translational modifications [48]. Previous research has identified

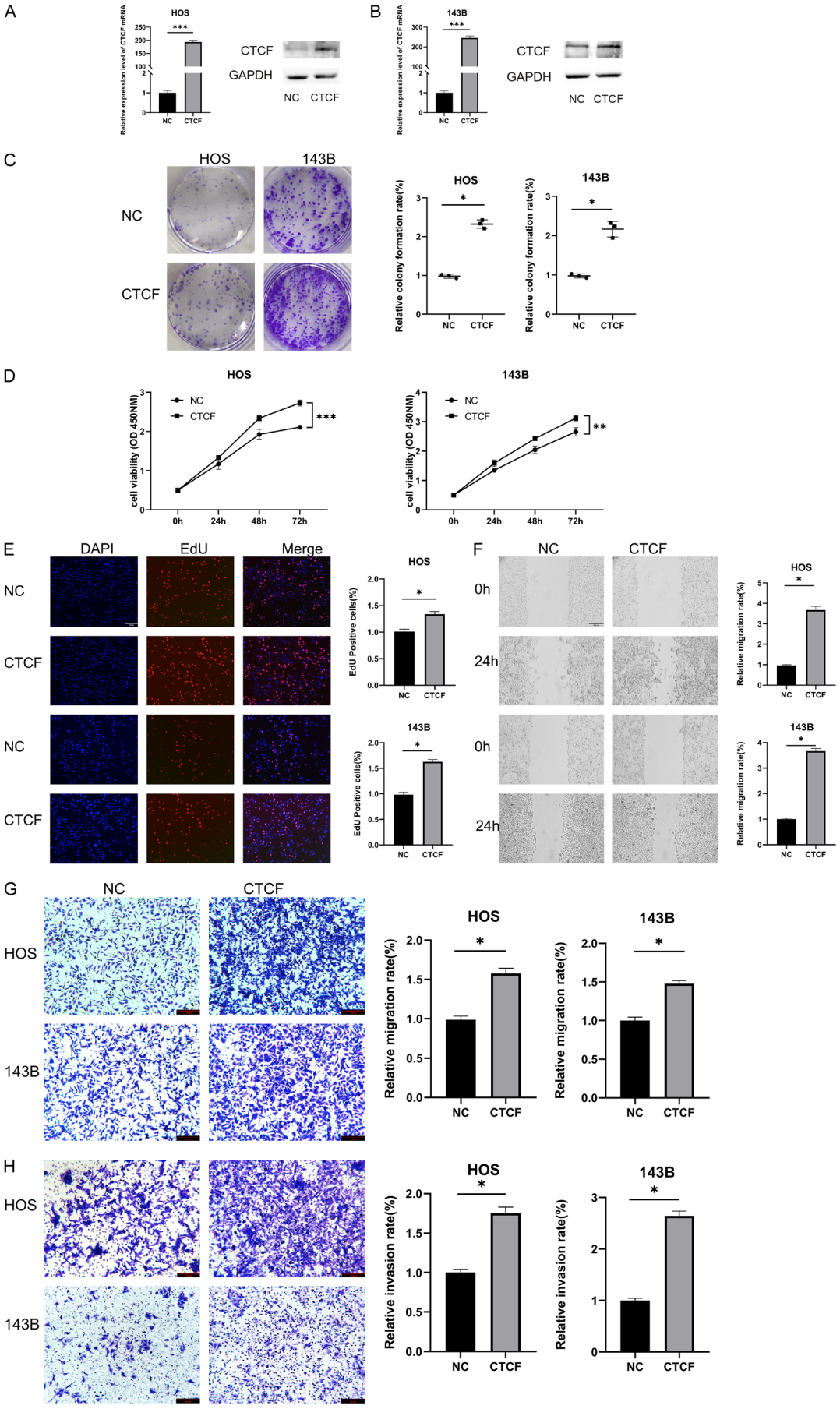

A
HOS
***
Relative expression level of CTCF mRNA
NC
CTCF
CTCF
GAPDH
NC CTCF
B
143B
***
Relative expression level of CTCF mRNA
NC
CTCF
CTCF
GAPDH
NC CTCF
C
HOS
143B
NC
CTCF
HOS
*
Relative colony formation rate(%)
NC
CTCF
143B
*
Relative colony formation rate(%)
NC
CTCF
D
HOS
NC
CTCF
***
cell viability (OD 450NM)
0h
24h
48h
72h
143B
NC
CTCF
**
cell viability (OD 450NM)
0h
24h
48h
72h
E
DAPI
EdU
Merge
NC
CTCF
NC
CTCF
HOS
*
EdU Positive cells(%)
NC
CTCF
143B
*
EdU Positive cells(%)
NC
CTCF
F
NC
CTCF
0h
24h
0h
24h
HOS
*
Relative migration rate(%)
NC
CTCF
143B
*
Relative migration rate(%)
NC
CTCF
G
NC
CTCF
HOS
143B
HOS
*
Relative migration rate(%)
NC
CTCF
143B
*
Relative migration rate(%)
NC
CTCF
H
HOS
143B
HOS
*
Relative invasion rate(%)
NC
CTCF
143B
*
Relative invasion rate(%)
NC
CTCF

Figure 3. Overexpression of CTCF enhances the proliferation, migration, and invasion of osteosarcoma cells. Overexpression efficiency of CTCF in HOS (A) and 143B (B) cells detected by qRT-PCR and WB. The effect of CTCF overexpression on the proliferative ability of osteosarcoma cells was detected by cell plate cloning, CCK-8 assay, and EdU assay. Scale bar = 100 μm (C-E). The effect of CTCF overexpression on migration and invasion abilities of osteosarcoma cells was detected by cell scratch assay (F) and Transwell migration and invasion assay (G, H). The scale bar in the cell scratch test is 100 μm. The scale bar in the Transwell migration and invasion assay is 50 and 100 μm. (Results are displayed as the mean ± SD, ***P < 0.001, **P < 0.01, *P < 0.05, n = 3).

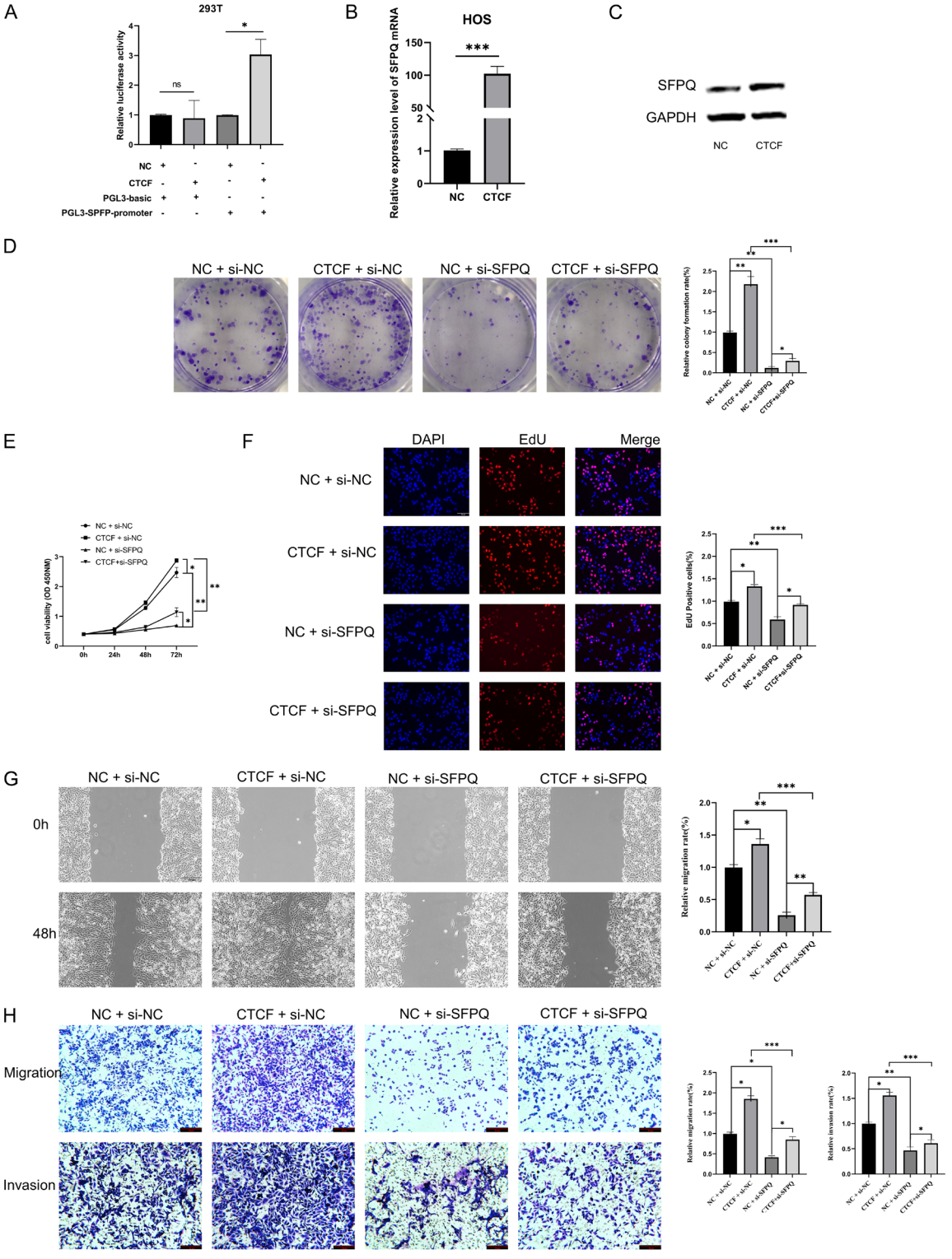


Figure 4. CTCF affects the biological behavior of osteosarcoma cells by regulating SFPQ. Dual luciferase activity detection (A). Expression levels of SFPQ were detected by qRT-PCR and WB in HOS cells after overexpression of CTCF. The band size of SFPQ is 100 kDa (B, C). Changes in the proliferative ability of HOS cells detected by rescue assay, cell plate assay, CCK-8 assay, and EdU assay. Scale bar = 100 μm (D-F). Changes in HOS cell migration and invasion ability detected by rescue assay, cell scratch assay, and Transwell assay (G, H). The scale bar in the cell scratch test is 100 μm. The scale bar in the Transwell assay is 50 μm. (Results are displayed as the mean ± SD, ***P < 0.001, **P < 0.01, *P < 0.05, n = 3).

CTCF as an oncogene in gastric and prostate tumors [49, 50] and reported its role as a transcriptional activator of genes [47, 51, 52]. However, its expression level and functional impact on osteosarcoma remained unclear.

In this study, we provided substantial experimental evidence that CTCF regulates the transcription of SFPQ in osteosarcoma cells. First, bioinformatic screening identified CTCF as a potential transcription factor for SFPQ. Subsequent qRT-PCR and western blot (WB) analyses confirmed that CTCF is highly expressed in osteosarcoma cells. To further investigate the functional role of CTCF, we conducted both knockdown experiments using specific CTCF siRNA and overexpression experiments using a CTCF plasmid. The results revealed that overexpression of CTCF enhanced, while knockdown reduced, the invasion, migration, and proliferation capacities of osteosarcoma cells, indicating that CTCF promotes the malignant behavior of these cells.

Furthermore, using a dual-luciferase reporter assay, we demonstrated that transfection with a CTCF overexpression plasmid significantly increased the relative luciferase activity of cells transfected with a reporter gene vector. This finding confirms the presence of a CTCF binding site within the SFPQ promoter sequence and establishes a regulatory relationship between CTCF and SFPQ. Finally, rescue experiments showed that the enhancement of osteosarcoma cell proliferation, motility, and invasion induced by CTCF overexpression could be reversed by SFPQ knockdown.

Overall, our study elucidates the link between CTCF and SFPQ, demonstrating that CTCF transcriptionally activates SFPQ in osteosarcoma cells, thereby enhancing their proliferative, migratory, and invasive capacities.

Although these results provide significant insight into the CTCF-SFPQ axis, they are based on *in vitro* experiments. Further investigation is required to verify the malignant regulatory function of the CTCF-SFPQ axis *in vivo*. Additionally, it is necessary to collect clinical blood specimens and tumor tissues from osteosarcoma patients to assess the efficacy of targeting the CTCF-SFPQ axis for clinical diagnosis and treatment.

Conclusion

CTCF expression was elevated in osteosarcoma. *In vitro*, increased CTCF expression markedly enhanced the proliferation, migration, and invasion of osteosarcoma cells. Mechanistically, CTCF appears to act as an oncogene, possibly through the transcriptional activation of SFPQ. This finding provides a novel strategy for targeting the CTCF/SFPQ axis in both academic research and clinical therapy for osteosarcoma.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81601931; 81672229), the Natural Science Foundation of Jiangsu Province (BK20150475), the Youth Medical Key Talent Project of Jiangsu (QNRC2016844), the “Six One Projects” for high-level health professionals in Jiangsu Province Top Talent Project (LGY2019089), the Jiangsu Provincial key research and development program (BE2020679), the Shenzhen Science and Technology Program (No. KQTD20170810154011370), the Medical education collaborative innovation fund of Jiangsu University (JDY-2022001), and the Zhenjiang City social development project (SH2023018).

Disclosure of conflict of interest

None.

Address correspondence to: Xinyu Zhong, Affiliated Hospital of Jiangsu University, 438 Jiefang Road, Zhenjiang 212001, Jiangsu, China. E-mail: rainzxy805@hotmail.com; Qiping Zheng, Depart-

ment of Laboratory Medicine, School of Medicine, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, Jiangsu, China. E-mail: qp_zheng@hotmail.com; Wenchao Zhang, Affiliated Jintan Hospital of Jiangsu University, Changzhou 213200, Jiangsu, China. E-mail: beny0731@126.com

References

[1] Kager L, Tamamyan G and Bielack S. Novel insights and therapeutic interventions for pediatric osteosarcoma. Future Oncol 2017; 13: 357-368.

[2] Shoaib Z, Fan TM and Irudayaraj JMK. Osteosarcoma mechanobiology and therapeutic targets. Br J Pharmacol 2022; 179: 201-217.

[3] Yang C, Tian Y, Zhao F, Chen Z, Su P, Li Y and Qian A. Bone microenvironment and osteosarcoma metastasis. Int J Mol Sci 2020; 21: 6985.

[4] Brown HK, Tellez-Gabriel M and Heymann D. Cancer stem cells in osteosarcoma. Cancer Lett 2017; 386: 189-195.

[5] Wadhwa N. Osteosarcoma: diagnostic dilemmas in histopathology and prognostic factors. Indian J Orthop 2014; 48: 247-254.

[6] Kumar R, Kumar M, Malhotra K and Patel S. Primary osteosarcoma in the elderly revisited: current concepts in diagnosis and treatment. Curr Oncol Rep 2018; 20: 13.

[7] Yang L, Gilbertsen A, Jacobson B, Pham J, Fujioka N, Henke CA and Kratzke RA. SFPQ and its isoform as potential biomarker for non-small-cell lung cancer. Int J Mol Sci 2023; 24: 12500.

[8] Kikhai T, Agapkina Y, Silkina M, Prikazchikova T and Gottikh M. The cellular SFPQ protein as a positive factor in the HIV-1 integration. Biochimie 2024; 222: 9-17.

[9] Kok VJT, Tang JY, Eng GWL, Tan SY, Chin JTF, Quek CH, Lai WX, Lim TK, Lin Q, Chua JJE and Cheong JK. SFPQ promotes RAS-mutant cancer cell growth by modulating 5'-UTR mediated translational control of CK1α. NAR Cancer 2022; 4: zcac027.

[10] Pashintseva NV, Shishkin SS, Lisitskaya KV, Kovalev LI, Kovaleva MA, Eryomina LS, Kamenikhina IA, Novikova LA and Sadykhov EG. Study of splicing factor, proline- and glutamine-rich by proteomic techniques in human malignant and nonmalignant cell lines. Protein Pept Lett 2016; 23: 958-966.

[11] Mao L, Jiang P, Lei X, Zhang B, Zhong X, Yin Z, Wang Y, Li D, Zhang Y and Zheng Q. The splicing factor proline and glutamine rich promotes the growth of osteosarcoma via the c-Myc signaling pathway. Am J Cancer Res 2023; 13: 2488-2503.

[12] Dehingia B, Milewska M, Janowski M and Pękowska A. CTCF shapes chromatin structure and gene expression in health and disease. EMBO Rep 2022; 23: e55146.

[13] Wang H, Maurano MT, Qu H, Varley KE, Gertz J, Pauli F, Lee K, Canfield T, Weaver M, Sandstrom R, Thurman RE, Kaul R, Myers RM and Stamatoyannopoulos JA. Widespread plasticity in CTCF occupancy linked to DNA methylation. Genome Res 2012; 22: 1680-1688.

[14] Zhang H, Shi Z, Banigan EJ, Kim Y, Yu H, Bai XC and Finkelstein IJ. CTCF and R-loops are boundaries of cohesin-mediated DNA looping. Mol Cell 2023; 83: 2856-2871.e2858.

[15] Segueni J and Noordermeer D. CTCF: a misguided jack-of-all-trades in cancer cells. Comput Struct Biotechnol J 2022; 20: 2685-2698.

[16] Wei Z, Wang S, Xu Y, Wang W, Soares F, Ahmed M, Su P, Wang T, Orouji E, Xu X, Zeng Y, Chen S, Liu X, Jia T, Liu Z, Du L, Wang Y, Chen S, Wang C, He HH and Guo H. MYC reshapes CTCF-mediated chromatin architecture in prostate cancer. Nat Commun 2023; 14: 1787.

[17] Kemp CJ, Moore JM, Moser R, Bernard B, Teater M, Smith LE, Rabaia NA, Gurley KE, Guinney J, Busch SE, Shaknovich R, Lobanenkov VV, Liggitt D, Shmulevich I, Melnick A and Filippova GN. CTCF haploinsufficiency destabilizes DNA methylation and predisposes to cancer. Cell Rep 2014; 7: 1020-1029.

[18] Oh S, Oh C and Yoo KH. Functional roles of CTCF in breast cancer. BMB Rep 2017; 50: 445-453.

[19] Zhang B, Zhang Y, Zou X, Chan AW, Zhang R, Lee TK, Liu H, Lau EY, Ho NP, Lai PB, Cheung YS, To KF, Wong HK, Choy KW, Keng VW, Chow LM, Chan KK, Cheng AS and Ko BC. The CCCTC-binding factor (CTCF)-forkhead box protein M1 axis regulates tumour growth and metastasis in hepatocellular carcinoma. J Pathol 2017; 243: 418-430.

[20] Guo Y, Perez AA, Hazelett DJ, Coetzee GA, Rhie SK and Farnham PJ. CRISPR-mediated deletion of prostate cancer risk-associated CTCF loop anchors identifies repressive chromatin loops. Genome Biol 2018; 19: 160.

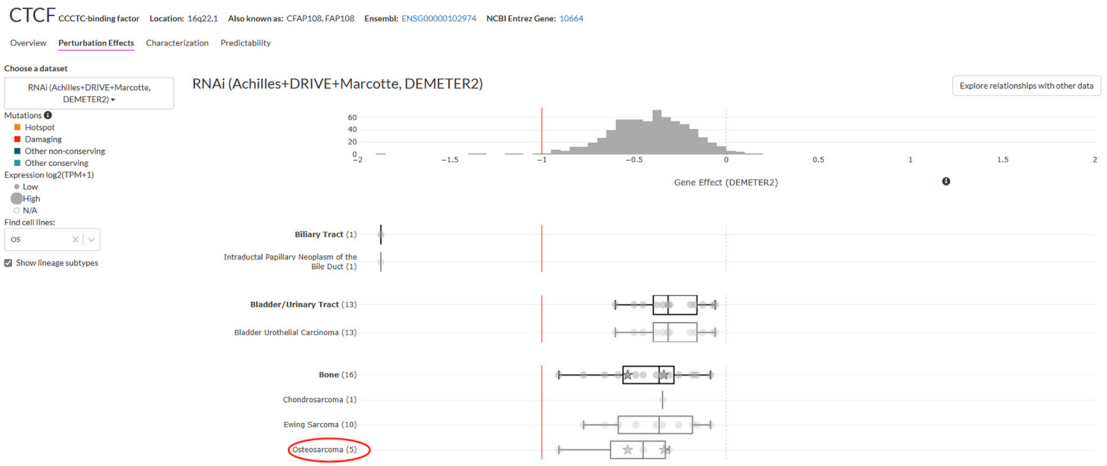
[21] Eldholm V, Haugen A and Zienolddiny S. CTCF mediates the TERT enhancer-promoter interactions in lung cancer cells: identification of a novel enhancer region involved in the regulation of TERT gene. Int J Cancer 2014; 134: 2305-2313.

[22] Zhan H, Xiao J, Wang P, Mo F, Li K, Guo F, Yu X, Liu X, Zhang B, Dai M and Liu H. Exosomal CTCF confers cisplatin resistance in osteosarcoma by promoting autophagy via the IGF2-AS/miR-579-3p/MSH6 Axis. J Oncol 2022; 2022: 9390611.

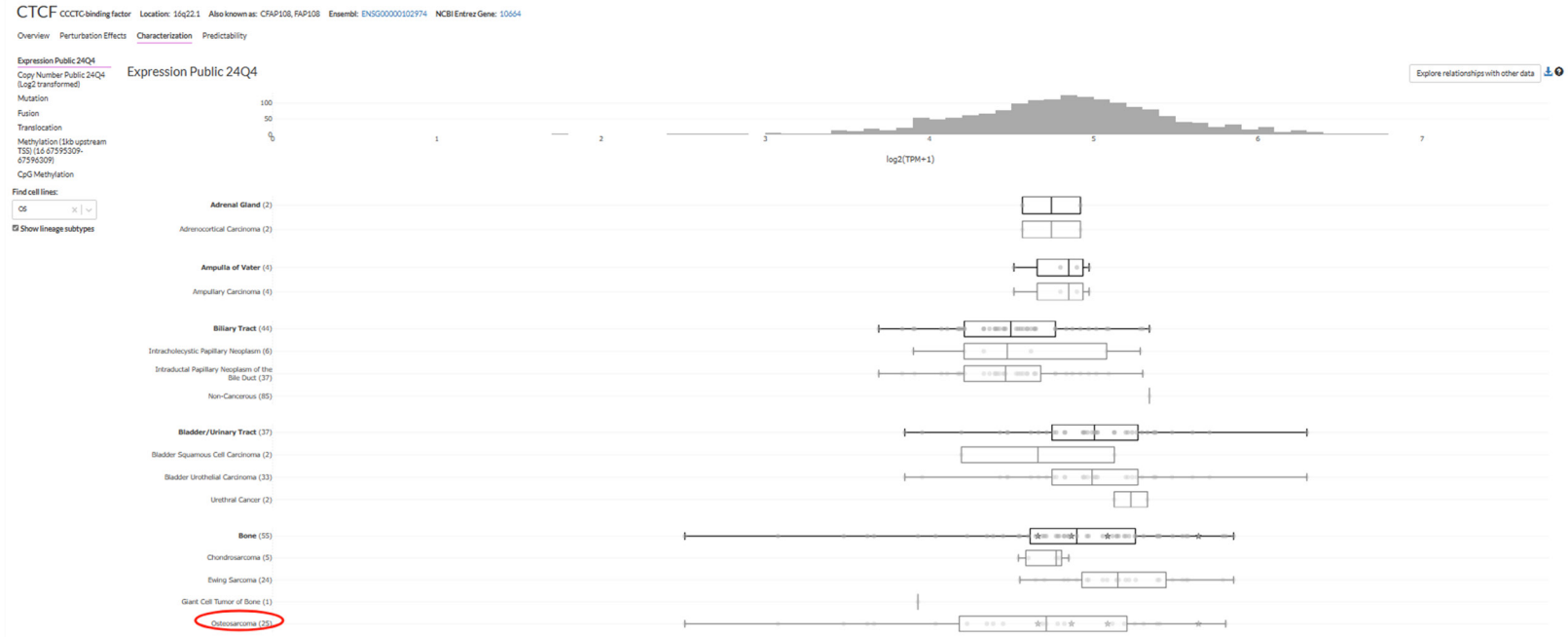
[23] Yoshida A. Osteosarcoma: old and new challenges. Surg Pathol Clin 2021; 14: 567-583.
[24] Li S, Zhang H, Liu J and Shang G. Targeted therapy for osteosarcoma: a review. J Cancer Res Clin Oncol 2023; 149: 6785-6797.
[25] Sadykova LR, Ntekim AI, Muyangwa-Semenova M, Rutland CS, Jeyapalan JN, Blatt N and Rizvanov AA. Epidemiology and risk factors of osteosarcoma. Cancer Invest 2020; 38: 259-269.
[26] Lindsey BA, Markel JE and Kleinerman ES. Osteosarcoma overview. Rheumatol Ther 2017; 4: 25-43.
[27] Belayneh R, Fourman MS, Bhogal S and Weiss KR. Update on osteosarcoma. Curr Oncol Rep 2021; 23: 71.
[28] Han G, Bi WZ, Xu M, Jia JP and Wang Y. Amputation versus limb-salvage surgery in patients with osteosarcoma: a meta-analysis. World J Surg 2016; 40: 2016-2027.
[29] Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jürgens H and Winkler K. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. J Clin Oncol 2002; 20: 776-790.
[30] Tian Z, Yang G, Jiang P, Zhang L, Wang J and Sun S. Long non-coding RNA Sox4 promotes proliferation and migration by activating Wnt/β-catenin signaling pathway in osteosarcoma. Pharmazie 2017; 72: 537-542.
[31] Min X, Heng H, Yu HL, Dan M, Jie C, Zeng Y, Ning H, Liu ZG, Wang ZY and Lin W. Anticancer effects of 10-hydroxycamptothecin induce apoptosis of human osteosarcoma through activating caspase-3, p53 and cytochrome c pathways. Oncol Lett 2018; 15: 2459-2464.
[32] Gorlick R. Current concepts on the molecular biology of osteosarcoma. Cancer Treat Res 2009; 152: 467-478.
[33] Hua Y, Jia X, Sun M, Zheng L, Yin L, Zhang L and Cai Z. Plasma membrane proteomic analysis of human osteosarcoma and osteoblastic cells: revealing NDRG1 as a marker for osteosarcoma. Tumour Biol 2011; 32: 1013-1021.
[34] Pellarin I, Dall'Acqua A, Gambelli A, Pellizzari I, D'Andrea S, Sonego M, Lorenzon I, Schiappacassi M, Belletti B and Baldassarre G. Splicing factor proline- and glutamine-rich (SFPQ) protein regulates platinum response in ovarian cancer-modulating SRSF2 activity. Oncogene 2020; 39: 4390-4403.
[35] de Silva HC, Lin MZ, Phillips L, Martin JL and Baxter RC. IGFBP-3 interacts with NONO and SFPQ in PARP-dependent DNA damage repair in triple-negative breast cancer. Cell Mol Life Sci 2019; 76: 2015-2030.
[36] Ji Q, Zhang L, Liu X, Zhou L, Wang W, Han Z, Sui H, Tang Y, Wang Y, Liu N, Ren J, Hou F and Li Q. Long non-coding RNA MALAT1 promotes tumour growth and metastasis in colorectal cancer through binding to SFPQ and releasing oncogene PTBP2 from SFPQ/PTBP2 complex. Br J Cancer 2014; 111: 736-748.
[37] Klotz-Noack K, Klinger B, Rivera M, Bublitz N, Uhlitz F, Riemer P, Lüthen M, Sell T, Kasack K, Gastl B, Ispasanie SSS, Simon T, Janssen N, Schwab M, Zuber J, Horst D, Blüthgen N, Schäfer R, Morkel M and Sers C. SFPQ depletion is synthetically lethal with BRAF(V600E) in colorectal cancer cells. Cell Rep 2020; 32: 108184.
[38] Takayama KI, Suzuki T, Fujimura T, Yamada Y, Takahashi S, Homma Y, Suzuki Y and Inoue S. Dysregulation of spliceosome gene expression in advanced prostate cancer by RNA-binding protein PSF. Proc Natl Acad Sci U S A 2017; 114: 10461-10466.
[39] Hua JT, Ahmed M, Guo H, Zhang Y, Chen S, Soares F, Lu J, Zhou S, Wang M, Li H, Larson NB, McDonnell SK, Patel PS, Liang Y, Yao CQ, van der Kwast T, Lupien M, Feng FY, Zoubeidi A, Tsao MS, Thibodeau SN, Boutros PC and He HH. Risk SNP-mediated promoter-enhancer switching drives prostate cancer through lncRNA PCAT19. Cell 2018; 174: 564-575, e518.
[40] Gao Z, Chen M, Tian X, Chen L, Chen L, Zheng X, Wang H, Chen J, Zhao A, Yao Q, Zhu Q, Jin S, Hu H, Zeng S and Yu L. A novel human lncRNA SANT1 cis-regulates the expression of SLC47A2 by altering SFPQ/E2F1/HDAC1 binding to the promoter region in renal cell carcinoma. RNA Biol 2019; 16: 940-949.
[41] Hu L, Tang J, Huang X, Zhang T and Feng X. Hypoxia exposure upregulates MALAT-1 and regulates the transcriptional activity of PTB-associated splicing factor in A549 lung adenocarcinoma cells. Oncol Lett 2018; 16: 294-300.
[42] Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, Chen X, Taipale J, Hughes TR and Weirauch MT. The human transcription factors. Cell 2018; 172: 650-665.
[43] Huilgol D, Venkataramani P, Nandi S and Bhattacharjee S. Transcription factors that govern development and disease: an achilles heel in cancer. Genes (Basel) 2019; 10: 794.
[44] Wulfridge P, Yan Q, Rell N, Doherty J, Jacobson S, Offley S, Deliard S, Feng K, Phillips-Cremins JE, Gardini A and Sarma K. G-quadruplexes associated with R-loops promote CTCF binding. Mol Cell 2023; 83: 3064-3079, e3065.
[45] Debaugny RE and Skok JA. CTCF and CTCFL in cancer. Curr Opin Genet Dev 2020; 61: 44-52.

[46] Sun G, Wei Y, Zhou B, Wang M, Luan R, Bai Y, Li H, Wang S, Zheng D, Wang C, Wang S, Zeng K, Liu S, Lin L, He M, Zhang Q and Zhao Y. BAP18 facilitates CTCF-mediated chromatin accessible to regulate enhancer activity in breast cancer. Cell Death Differ 2023; 30: 1260-1278.
[47] Fang C, Wang Z, Han C, Safgren SL, Helmin KA, Adelman ER, Serafin V, Basso G, Eagen KP, Gaspar-Maia A, Figueroa ME, Singer BD, Ratan A, Ntziachristos P and Zang C. Cancer-specific CTCF binding facilitates oncogenic transcriptional dysregulation. Genome Biol 2020; 21: 247.
[48] Docquier F, Kita GX, Farrar D, Jat P, O'Hare M, Chernukhin I, Gretton S, Mandal A, Alldridge L and Klenova E. Decreased poly (ADP-ribosyl) ation of CTCF, a transcription factor, is associated with breast cancer phenotype and cell proliferation. Clin Cancer Res 2009; 15: 5762-5771.
[49] Shan Z, Li Y, Yu S, Wu J, Zhang C, Ma Y, Zhuang G, Wang J, Gao Z and Liu D. CTCF regulates the FoxO signaling pathway to affect the progression of prostate cancer. J Cell Mol Med 2019; 23: 3130-3139.
[50] Yu L, Gao Y, Ji B, Feng Z, Li T and Luan W. CTCF-induced upregulation of LINC01207 promotes gastric cancer progression via miR-1301-3p/PODXL axis. Dig Liver Dis 2021; 53: 486-495.
[51] Beishline K, Vladimirova O, Tutton S, Wang Z, Deng Z and Lieberman PM. CTCF driven TERRA transcription facilitates completion of telomere DNA replication. Nat Commun 2017; 8: 2114.
[52] Sekiya T, Kato K, Kawaguchi A and Nagata K. Involvement of CTCF in transcription regulation of EGR1 at early G1 phase as an architecture factor. Sci Rep 2019; 9: 329.

A



B



Supplementary Figure 1. Gene effect score of CTCF in osteosarcoma cells is significantly less than -1 (A). CTCF is in a high expression state in all osteosarcoma cells (B).