

# Histone methyltransferase SUV39H2 serves oncogenic roles in osteosarcoma

LIANHUA PIAO<sup>1\*</sup>, XIAOFENG YUAN<sup>2\*</sup>, MING ZHUANG<sup>2</sup>, XUBIN QIU<sup>2</sup>,  
XIAOSHUANG XU<sup>1</sup>, REN KONG<sup>1</sup> and ZHIWEI LIU<sup>2</sup>

<sup>1</sup>Institute of Bioinformatics and Medical Engineering, Jiangsu University of Technology;

<sup>2</sup>Department of Orthopaedics, The Third Affiliated Hospital of Soochow University,  
Changzhou, Jiangsu 213000, P.R. China

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**Abstract.** Suppressor of variegation 3-9 homologue 2 (SUV39H2), a SET domain-containing histone methyltransferase, trimethylates histone H3 at lysine 9 and serves crucial roles in heterochromatin organization and genome stability. SUV39H2 is overexpressed in various types of human cancer, whereas it is almost undetectable in normal adult tissues, except testis. The aim of this study was to investigate a potential role of SUV39H2 in osteosarcoma. In the present study, increased SUV39H2 expression levels were observed in osteosarcoma, the most common primary bone cancer in children and adolescents, and the knockdown of SUV39H2 expression by specific small interfering RNAs in osteosarcoma cells markedly suppressed cancer cell growth and led to a notable reduction in cell viability. Furthermore, overexpression of SUV39H2 promoted cell proliferation, which indicated that SUV39H2 may possess oncogenic activity in human osteosarcoma. Notably, depletion of SUV39H2 expression caused an increase in the population of G1 phase and induced apoptosis, which implied that SUV39H2 may have biological significance in the process of cell cycle. These results indicated that SUV39H2 may be an ideal target for osteosarcoma therapeutics.

## Introduction

Osteosarcoma is the most common malignant primary bone tumor in children and young adults (1). Currently, the 5-year overall survival rate for patients with non-metastatic

osteosarcoma is 60-70%, whereas the survival rate for patients with metastatic osteosarcoma is 20-30% (2). At present, the combination of pre- and postoperative chemotherapy with surgical resection is the main treatment for osteosarcoma, and the majority of patients commence similar chemotherapy schedules following diagnosis. There is currently no active agent for the treatment of osteosarcoma, therefore, to refine treatment strategies for osteosarcoma it is necessary to improve our understanding of osteosarcoma etiology and to identify novel targetable agents as adjuvant to chemotherapeutics to improve patient outcomes.

Osteosarcoma is characterized by numerical chromosomal instability (3). A recent study demonstrated that histone methylation, a mechanism for modifying chromatin structure, contributes to aberrant transcriptional regulation and oncogenic signaling pathways in osteosarcoma (4). Furthermore, increased expression of histone methyltransferases (HMTs), including G9a, enhancer of zeste homolog 2 (EZH2), nuclear SET domain-containing 3 (NSD3), protein arginine N-methyltransferase 1, and coactivator-associated arginine methyltransferase 1, contribute to osteosarcoma development (5-10). These data suggested that genes encoding histone methylation modifiers, HMTs and histone demethylases (HDMTs), may serve key roles in the development and progression of OS.

Suppressor of variegation 3-9 homologue 2 (SUV39H2; also known as lysine N-methyltransferase 2) is a member of the SET domain-containing HMT family that specifically trimethylates lysine 9 of histone H3 (H3K9me3) (11), which is a type of post-translational modification that is associated with transcriptional repression and heterochromatin formation (12,13). SUV39H2 shares 58% identity with SUV39H1, another H3K9 methyltransferase belonging to the same protein family. In adult tissues, SUV39H2 expression is restricted to the testis, whereas its expression is largely present in embryonic stem cells (14,15). Notably, SUV39H1- or SUV39H2-deficient mice exhibit normal viability, whereas SUV39H<sup>-/-</sup> null mice exhibit chromosomal instabilities with abnormally long telomeres and display severely reduced viability (16,17). SUV39H2 appears to be highly upregulated in cervical, lung, prostate, bladder and esophageal cancer, as well as acute lymphoblastic leukemia (18-20). The function of SUV39H2 in

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*Correspondence to:* Dr Zhiwei Liu, Department of Orthopaedics, The Third Affiliated Hospital of Soochow University, 185 Juqian Street, Changzhou, Jiangsu 213000, P.R. China  
E-mail: lzwei117@163.com

\*Contributed equally

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cancer remains to be fully elucidated; however, it is partially dependent on the regulation of H3K9 methylation status. A limited number of studies have indicated that SUV39H2 may be associated with cellular senescence through the methylation of H3K9 (21) and may be involved in the DNA repair pathway through methylation of histone H2AX at K134 (18). Therefore, cells overexpressing SUV39H2 are more resistant to chemotherapy (19). SUV39H2 has also been reported to interact with androgen receptor and melanoma-associated antigen 11, and to increase androgen-dependent transcriptional activity (22). Our previous studies demonstrated that the SUV39H2-dependent methylation of lysine-specific histone demethylase (LSD)-1 at K322 leads to stabilization of LSD1 protein through the inhibition of its ubiquitination (23) and that automethylation of SUV39H2 impairs binding affinities to substrate proteins such as histone H3 and LSD1 (24).

In the present study, SUV39H2 expression was demonstrated to be upregulated in human osteosarcoma cell lines and may promote osteosarcoma cancer growth. In addition, reduced SUV39H2 expression led to the suppression of cancer cell growth through the induction of G1 phase arrest and increased rates of apoptosis, which suggested that SUV39H2 may contribute to the development and progression of osteosarcoma, and may be a novel targetable gene for the treatment of patients with osteosarcoma.

## Materials and methods

**Cell line and cell culture.** The 293T cell line, the human osteoblast cell line hFOB1.19, and the human osteosarcoma cell lines HOS, U2OS and MG-63 were purchased from The Shanghai Institute for Biological Sciences, Chinese Academy of Cell Resource Center (Shanghai, China). HOS, U2OS, and MG-63 were grown in minimum essential medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA); 293T and hFOB1.19 cells were grown in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.), supplemented with 10% fetal bovine serum (ScienCell Research Laboratories, Inc., San Diego, CA, USA) and 1% antibiotic/antimycotic solution (Gibco; Thermo Fisher Scientific, Inc.) at 37°C in a humidified incubator with 5% CO<sub>2</sub>.

**Stable cell line generation.** Approximately 40-50% confluent cells were transfected with pCAGGS-SUV39H2-3xFlag (SUV39H2)-overexpression or pCAGGS-3xFlag (Mock) vectors using FuGENE<sup>®</sup> 6 Transfection Reagent (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. Briefly, 6  $\mu$ l FuGENE<sup>®</sup> 6 Transfection Reagent was added to 100  $\mu$ l Opti-MEM I reduced-serum medium (Gibco; Thermo Fisher Scientific, Inc.) and incubated for 5 min at room temperature. Plasmid DNA (1  $\mu$ g/ $\mu$ l; 2  $\mu$ g) was added to FuGENE6 Transfection Reagent/Opti-MEM, and the mixture was incubated for 15 min at room temperature followed by adding to each well of a 6-well plate. To obtain stably expressing cells, 293T cells transfected with SUV39H2-overexpression or Mock vectors were subsequently selected with geneticin (0.4 mg/ml).

**RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** Total RNA was isolated from

1x10<sup>6</sup> cells with EASYspin Plus Tissue/Cell RNA Extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China). Extracted RNA was reverse transcribed to cDNA with ThermoScript First-Strand cDNA Synthesis kit (Aidlab Biotechnologies Co., Ltd.), according to the manufacturer's protocol. qPCR was performed using SYBR Premix *Ex Taq* (Takara Biotechnology Co., Ltd., Dalian, China) and the ABI StepOnePlus Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: Initial denaturation at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 34 sec. The primer sequences were: GAPDH forward, 5'-ATGGAAATCCCATCACCATCTT-3' and reverse 5'-CGCCCCACTTGATTTTGG-3'; and SUV39H2 forward, 5'-AATGGAAAGGATGGCCAGATT-3' and reverse, 5'-ACGGGCACTTCAGATTTTGC-3'. Each sample was analyzed in duplicate, and the relative gene expression levels were normalized to GAPDH expression levels and quantified using the comparative 2<sup>- $\Delta\Delta$ C<sub>q</sub></sup> method (25).

**Small interfering (si)RNA transfection.** SUV39H2-specific siRNA oligonucleotide duplexes (siSUV39H2#1, 5'-CACAG AUUGCUUCUUCAA-3'; siSUV39H2#2, 5'-CUGGAAUC AGCUUAGUCA-3') were synthesized by Biolino Nucleic Acid Technology Co., Ltd. (Beijing, China). siRNA against enhanced green fluorescent protein (siEGFP; 5'-GCAGCAC GACUUCUUAAG-3') and si-negative control (siNC; 5'-UUC UCCGAACGUGUCACGUTT-3') were used as control siRNAs. Osteosarcoma cells with the density of 60-70% were transfected with siSUV39H2, siEGFP or siNC (100 nM) using Lipofectamine<sup>®</sup> RNAiMAX (Thermo Fisher Scientific, Inc.) and Opti-MEM I reduced-serum medium (Gibco; Thermo Fisher Scientific, Inc.), according to the manufacturer's, and the transfected cells were incubated continuously at 37°C for 72-96 h for further experiments.

**RNA-sequencing (RNA-seq) analysis.** Indexed libraries from HOS cells incubated with siEGFP (control) or siSUV39H2 were subjected to RNA-seq on the Illumina Hiseq 2000 Sequencing platform (Illumina, Inc., San Diego, CA, USA). Total RNA was extracted as aforementioned, and the integrity of samples was confirmed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). Gene expression levels were quantified using the fragments per kilobase of transcript per million mapped reads (FPKM) normalization method (26), and Cufflinks v 2.2.1 (<http://cole-trapnell-lab.github.io/cufflinks/install>) was used for FPKM quantification. Gene expression and differential transcription between siEGFP- and siSUV39H2-treated cells were evaluated using Cuffdiff. The lists of significantly differentially expressed genes (DEGs) were obtained with the thresholds of P $\leq$ 0.05 and a fold-change  $\geq$ 2.

**Cell viability and colony-formation assays.** Cell proliferation assays were performed using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Shanghai, China), according to manufacturer's protocols. Cells (1x10<sup>4</sup> cells/well) were plated in 96-well plates the day before treatment to allow cell attachment and transfected with siRNAs, as aforementioned. Cell viability was measured 96 h following transfection by measuring the absorbance at a wavelength of 450 nm and

the relative cell viability was calculated as the percentage of absorption.

For colony-formation assays, cells were seeded (2,000 cells/well) in 6-well plates and treated with siRNAs two times, once every 4 days. After 14 days, the cells were washed with PBS 3 times, fixed with methanol for 30 min, and stained with 0.1% crystal violet solution for another 15-20 min at room temperature. After washing with PBS, the 6-well plates were dried and scanned. Mock and SUV39H2 stably overexpressing 293T cells (5,000 cells/well) were seeded in 60-mm cell culture dish and cultured for 7 days continuously at 37°C, and subsequently stained with 0.1% crystal violet as aforementioned. U2OS cells (500 cells/well) in 6-well plates were transfected with pCAGGS-SUV39H2-3xFlag (SUV39H2)-overexpression or pCAGGS-3xFlag (Mock) vectors using FuGENE6 Transfection Reagent two times, once every 3 days. Cells were stained with 0.1% crystal violet at day 10. To observe growth of Mock and SUV39H2 stably overexpressing 293T cells, equal amounts of cells (500 cells/well) were seeded into 96-well plate and incubated at 37°C. Relative viable cell numbers were evaluated every day for 7 days incubating with CCK-8 for 2 h at 37°C, and the absorbance was measured at a wavelength of 450 nm everyday.

**Western blot analysis.** Cells at 80-90% confluence were lysed in radioimmunoprecipitation assay buffer [50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% Nonidet-P40 and 0.1 mM PMSF] with complete protease inhibitor cocktail (Roche Applied Science, Penzberg, Germany). Protein concentrations were determined by Bicinchoninic Acid Assay, and 20 µg protein was loaded into each well and separated by 12% SDS-PAGE. The proteins were transferred onto polyvinylidene fluoride membranes and blocked with 5% milk in TBS + 0.1% Tween-20 buffer for 1 h at room temperature followed by the overnight incubation with primary antibodies at 4°C, and subsequent incubation with secondary antibodies for 1 h at room temperature. Protein bands were visualized using Tanon High-sig ECL Western Blotting Substrate (Tanon Science and Technology Co., Ltd., Shanghai, China), and the relative density of the protein band of interest was normalized to β-actin and quantified using Tanon Image Software v1.0 (Tanon Science and Technology Co., Ltd.). Anti-Flag (1:8,000; cat. no. F-7425; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), SUV39H2 polyclonal antibody (1:250; cat. no. PA5-11366; Thermo Fisher Scientific, Inc.) and anti-β-actin (1:10,000; cat. no. A5441, Sigma-Aldrich; Merck KGaA) were used.

**Cell cycle and apoptosis analysis.** Osteosarcoma cells at a density of 40-60% were transfected with siSUV39H2#1 or siEGFP, as aforementioned, and incubated for 72 h at 37°C. The transfected cells were fixed with 70% ethanol in PBS at 4°C, followed by incubation with 500 µl of PBS containing 0.5 mg of boiled RNase at 37°C for 30 min. Subsequently, cells were stained with 50 µg/ml propidium iodide (PI) and analyzed using a BD FACSAria flow cytometer (BD Pharmingen; BD Biosciences, Franklin Lakes, NJ, USA) to investigate cell cycle. The percentage of cells in each group was calculated using ModFit LT software (Verity Software House, v3.3.11). Osteosarcoma cells incubated with specific siRNA for 96 h

at 37°C were collected for apoptosis analysis. Apoptosis was analyzed using fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection kit (cat. no. BD#556547; BD Biosciences), following the manufacturer's protocol.

**Statistical analysis.** Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Comparisons between two groups were analyzed using an independent two-sample t-test (two-tailed); comparisons among multiple groups were analyzed by one-way analysis of variance followed by the least significant difference post hoc test. Experiments were performed in duplicate or triplicate, and results were presented as mean ± standard deviation, except for the RT-qPCR experiments, which are presented as the mean ± standard error of the mean. P<0.05 was considered to indicate a statistically significant difference.

## Results

**SUV39H2 expression is increased in human osteosarcoma cell lines.** A number of HMTs and HDMTs are key drivers of cancer development and progression (27,28). Therefore, the present study aimed to understand the contribution of HMTs and HDMTs to osteosarcoma. Among the dozens of examined HMTs and HDMTs identified, SUV39H2, previously reported to be upregulated in a set of human cancers (18-20), was confirmed to be upregulated in osteosarcoma in the present study. Total RNA was isolated from osteosarcoma cell lines and the relative expression levels of SUV39H2 mRNA and protein were detected by RT-qPCR and western blotting, respectively (Fig. 1A and B, respectively). Notably, the SUV39H2 mRNA and protein expression levels in all three osteosarcoma cell lines (HOS, U2OS and MG-63) were significantly higher compared with those in the normal osteoblast cell line hFOB1.19.

**siSUV39H2 transfection reduces osteosarcoma cell viability.** To examine the biological functions of SUV39H2 in human osteosarcoma, the expression of SUV39H2 mRNA was knocked down using two independent SUV39H2-specific siRNAs (siSUV39H2#1 and siSUV39H2#2) in HOS, U2OS and MG63 cells, which was confirmed by RT-qPCR at 72 h post-transfection (Fig. 2A). SUV39H2 mRNA expression levels were reduced by ~75% following transfection with siSUV39H2#1 or siSUV39H2#2 compared with expression levels in the respective siEGFP-transfected or siNC-transfected control cells.

Colony formation assays were performed to assess the roles of SUV39H2 on the proliferation of osteosarcoma cells. At 14 days post-transfection, siSUV39H2#1- and siSUV39H2#2-transfected cells formed notably fewer and smaller colonies compared with the respective siEGFP- or siNC-treated cells (Fig. 2B). The viability of siSUV39H2-transfected osteosarcoma cells was measured by CCK-8 at 96 h post-transfection; cells transfected with SUV39H2-specific siRNAs exhibited a significant decrease in cell viability (~75%) compared with cells transfected with siEGFP or siNC (Fig. 2C; P<0.05). In addition, morphological changes were observed in siSUV39H2#1 or siSUV39H2#2 under normal microscope (Fig. 2D); SUV39H2 depleted

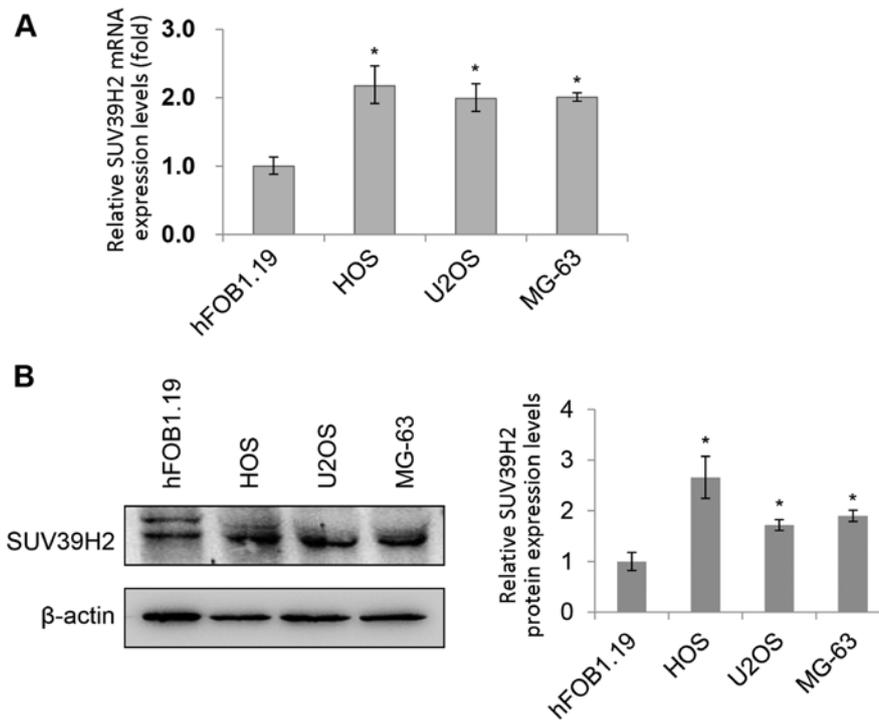


Figure 1. Expression of SUV39H2 is increased in osteosarcoma cells. (A) SUV39H2 mRNA expression was significantly higher in the osteosarcoma cell lines (HOS, U2OS and MG-63) compared with normal osteoblast cells (hFOB1.19), as detected by reverse transcription-quantitative polymerase chain reaction. GAPDH served as the loading control and used to normalize the expression data. Data are presented as the mean  $\pm$  standard error of the mean of two independent experiments; P-values were calculated by one-way analysis of the variance followed by least significant difference test; \* $P < 0.05$  vs. hFOB1.19. (B) SUV39H2 protein expression levels in osteosarcoma cell lines were examined by western blotting;  $\beta$ -actin was used as a loading control and for normalization. Data are presented as the mean  $\pm$  standard deviation of two independent experiments; \* $P < 0.05$  vs. hFOB1.19. SUV39H2, suppressor of variegation 3-9 homologue 2.

cells became large and angled. These results supported the potential oncogenic functions of SUV39H2 in the development and progression of osteosarcoma. To further determine whether the reduction in cell viability was due to induction of apoptosis, flow cytometric analysis of Annexin V-FITC/PI stained cells was performed on U2OS cells transfected with either siEGFP or with siSUV39H2#1 96 h. A higher proportion of Annexin V-FITC positive cells were detected in siSUV39H2-transfected cells compared with control siEGFP-treated cells ( $P < 0.05$ ; Fig. 3).

**Overexpression of SUV39H2 promotes cell growth.** To further investigate the roles of SUV39H2 in cells, attempts were made to produce hFOB1.19 cells that stably expressed SUV39H2. However, following selection with G418 (geneticin) for one month, the expression of SUV39H2 could not be detected by western blotting (data not shown). Subsequently, considering the issue of transfection efficiency (29), a 293T cell line was established that did stably express Flag-tagged SUV39H2, which was confirmed by western blot analysis (Fig. 4A). The SUV39H2-overexpression 293T cells exhibited increased growth rate compared with control Mock cells (Fig. 4B). Mock or SUV39H2-overexpressing cells were seeded at equal density (5,000 cells/60 mm dish) and allowed to proliferate for 7 days. SUV39H2 stably expressing cells exhibited larger and more numerous colonies compared with Mock cells (Fig. 4C). To assess the effects of SUV39H2 overexpression on osteosarcoma cell growth, SUV39H2 was transiently overexpressed

in U2OS cells; increased SUV39H2 expression promoted cell proliferation compared with Mock cells (Fig. 4D).

**SUV39H2 knockdown leads to G1 phase arrest in osteosarcoma cells.** As knockdown of SUV39H2 led to a reduction of cell viability and overexpression of SUV39H2 promoted cell proliferation, it was hypothesized that SUV39H2 may be involved in the process of cell cycle regulation. To assess the function of SUV39H2 in the progression of cell cycle, siSUV39H2#1- and siEGFP-transfected U2OS and MG-63 cells were collected, fixed with ethanol, stained with propidium iodide (PI) and analyzed using flow cytometry. The population of cells at the G1 phase in SUV39H2-depleted U2OS cells was significantly increased compared with that in siEGFP-treated cells (Fig. 5A). A similar increase in G1 population was observed in MG-63 osteosarcoma cells transfected with siSUV39H2#1 (Fig. 5B). These results indicated that depletion of SUV39H2 led to G1 cell cycle arrest and suggested that SUV39H2 may be involved in cell cycle regulation.

To identify genes associated with cell cycle that are also mediated by SUV39H2, RNA-seq gene expression analysis was performed in HOS cells treated with siSUV39H2#1 or siEGFP. A total of 438 DEGs, including a set of genes related to the G1/S cell cycle transition (DNA polymerase  $\epsilon 2$ , cyclin E1, origin recognition complex subunit 1, cyclin dependent kinase inhibitor 2D, proteasome inhibitor subunit 1 and RB-binding protein 8) were identified, supporting the possibility that SUV39H2 serves an essential role in cell cycle.

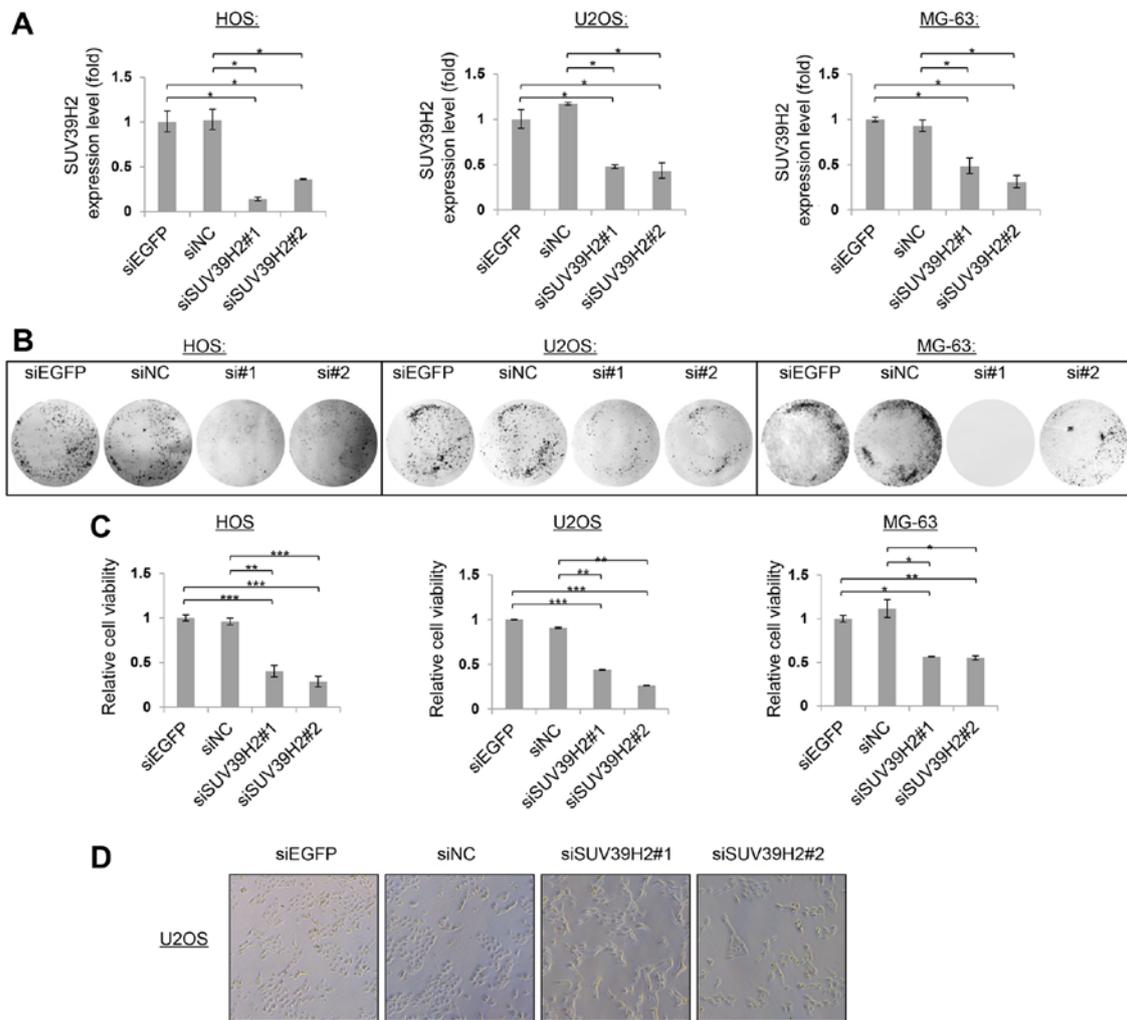


Figure 2. SUV39H2 knockdown decreases osteosarcoma cell viability. (A) Reverse transcription-quantitative polymerase chain reaction was used to examine SUV39H2 expression in HOS, U2OS and MG-63 osteosarcoma cells transfected with SUV39H2-specific siRNAs (siSUV39H2#1 and siSUV39H2#2) or control siRNAs (siEGFP and siNC) for 72 h. Data are presented as the mean  $\pm$  standard error of the mean of two independent experiments; \* $P < 0.05$  vs. siEGFP or siNC. (B) Colony-formation assays with osteosarcoma cells following SUV39H2 knockdown. Cells transfected with SUV39H2-specific siRNAs for 14 days were fixed with methanol and stained with 0.1% crystal violet. (C) SUV39H2-depleted osteosarcoma cancer cells exhibited a significant reduction in viability, as determined by CCK-8 assay assessed at 96 h post-transfection. Data are presented as the mean  $\pm$  standard deviation of three experimental repeats; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.005$  vs. siEGFP or siNC. (D) Morphology of U2OS cells following transfection with siEGFP, siNC, siSUV39H2#1 or siSUV39H2#2 (magnification,  $\times 200$ ). EGFP, enhanced green fluorescent protein; NC, negative control; si, small interfering RNA; SUV39H2, suppressor of variegation 3-9 homologue 2.

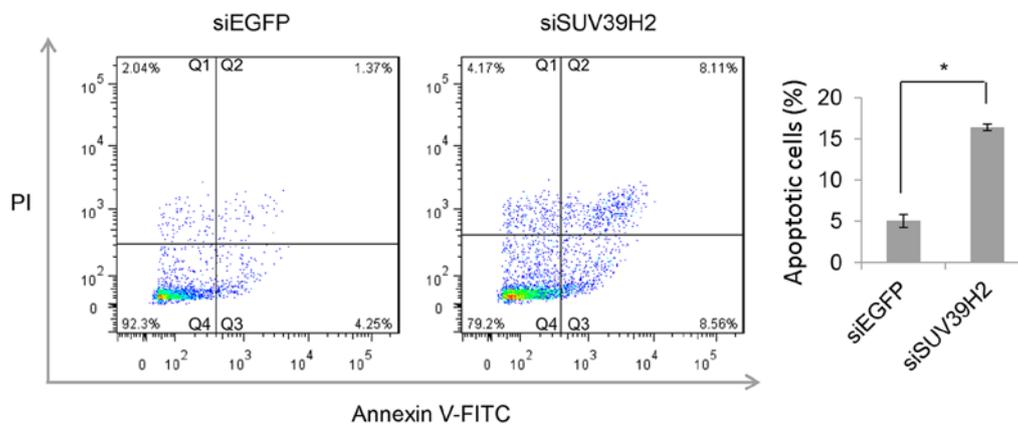


Figure 3. SUV39H2 knockdown induces apoptosis in U2OS osteosarcoma cells. Apoptosis was analyzed by Annexin V-FITC and PI staining in U2OS cells transfected with siEGFP or siSUV39H2. Q1 (FITC<sup>+</sup>/PI<sup>+</sup> stained cells) represents necrotic cells, Q2 (FITC<sup>+</sup>/PI<sup>-</sup>) represents late stage apoptotic cells, Q3 (FITC<sup>-</sup>/PI<sup>+</sup>) quadrant represents early stage apoptotic cells, and Q4 (FITC<sup>-</sup>/PI<sup>-</sup>) represents the live cells. A two-sample t-test (two-tailed) was used to determine the significance; data are presented as the mean  $\pm$  standard deviation \* $P < 0.05$ . EGFP, enhanced green fluorescent protein; FITC, fluorescein isothiocyanate; PI, propidium iodide; Q, quadrant; si, small interfering RNA; SUV39H2, suppressor of variegation 3-9 homologue 2.

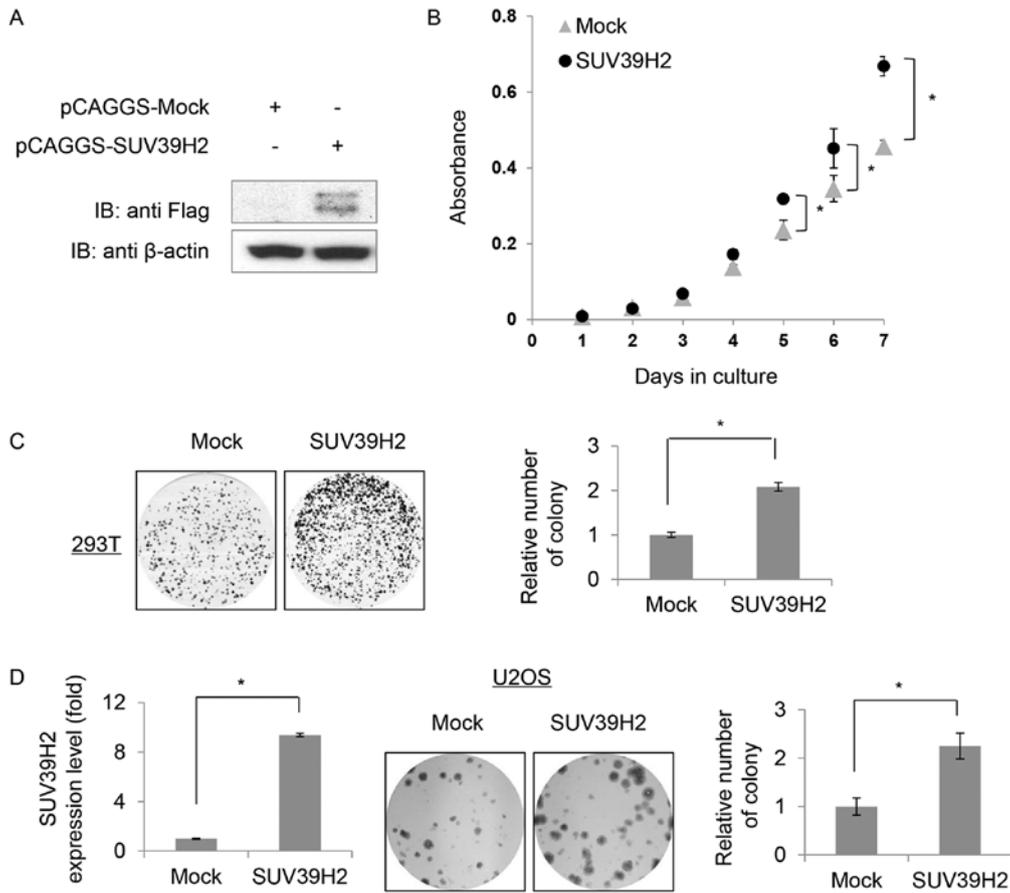


Figure 4. Overexpression of SUV39H2 promotes cell growth. (A) 293T cells were transfected with Mock or SUV39H2 expression vectors and the protein expression levels of SUV39H2 were measured by western blot analysis. (B) Mock or SUV39H2 stably expressing 293T cell viability by Cell Counting kit-8. Y-axis represents the absorbance of cells that represent relative viable cell number. Data are presented as the mean  $\pm$  SD of three independent experiments. Independent two-sample t-test (two-tailed) was used to calculate the statistical significance; \* $P < 0.05$ . (C) Representative images of colony-formation assay demonstrated that overexpression of SUV39H2 in 293T cells promoted cell growth. Data are presented as the mean  $\pm$  SD of three independent experiments; \* $P < 0.05$ . (D) Mock or SUV39H2 overexpression vector was transfected into U2OS cells twice at 72 h intervals, and cells were stained at day 10. SUV39H2 overexpression was confirmed by reverse transcription-quantitative polymerase chain reaction. Representative colony images are presented; these experiments were repeated twice and the average number of colonies from the two experiments was calculated. Data are presented as the mean  $\pm$  SD; \* $P < 0.05$ . SD, standard deviation; SUV39H2, suppressor of variegation 3-9 homologue 2.

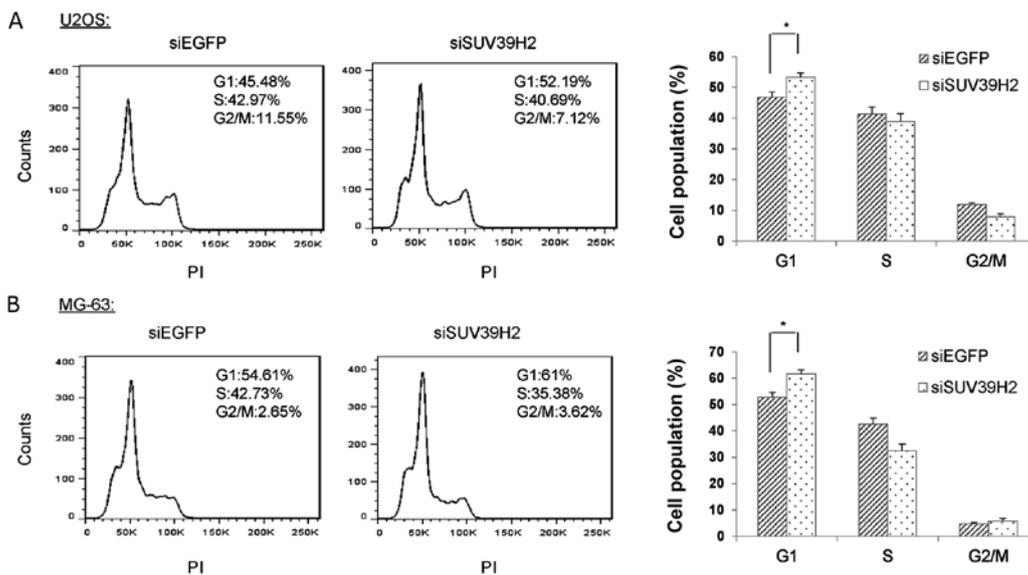


Figure 5. SUV39H2 knockdown leads to an increase in G1 phase cell population. (A and B) PI staining was used to examine cell cycle in (A) U2OS and (B) MG-63 cells treated with siSUV39H2 or siEGFP for 72 h. The average percent of the cells in each phase (G1, S and G2/M) of cell cycle from two independent experiments were calculated. Data are presented as the mean  $\pm$  standard deviation of two independent experiments; \* $P < 0.05$ . EGFP, enhanced green fluorescent protein; PI, propidium iodide; si, small interfering RNA; SUV39H2, suppressor of variegation 3-9 homologue 2.

## Discussion

Histone methylation serves key roles in normal mammalian development and in regulating gene expression (30,31). In addition to the importance of histone methylation in normal physiological functions, previous studies have indicated that its deregulation is deeply involved in the development of human cancer, and global levels of some histone methylation events were correlated with an increase of cancer recurrence and poor survival (27,28). In particular, recent studies suggested that aberrant expression of HMTs and HDMTs may serve crucial roles in human carcinogenesis (32,33).

A limited number of studies have indicated that it may be plausible that targeting abnormal HMTs and HDMTs in cancer cells may partly contribute to the treatment of osteosarcoma. For example, EZH2 methyltransferase, which trimethylates H3K27 serves oncogenic roles in human osteosarcoma (6), and treatment with EZH2 inhibitor leads to a marked reduction of cell viability in osteosarcoma (34); increased expression of the HMT NSD3 has been observed in osteosarcoma, and NSD3 is likely to serve key roles in the development of osteosarcoma (8). Another study reported that LSD1 is overexpressed in osteosarcoma, and treatment of osteosarcoma cells with the LSD1 inhibitor tranylcypromine reduced cell growth (35). Therefore, identification and clarification of the biological functions of aberrant histone methylation modifiers in osteosarcoma may attribute to identifying novel targetable genes and pathways in osteosarcoma.

SUV39H2 trimethylates H3K9, which is an important feature of cellular senescence and is essential for the viability of cells (12,13). *Suv39h1* and *Suv39h2* knockout mice are lethal, and exhibit abnormally long telomeres with reduced binding to the chromobox (Cbx) proteins Cbx1, Cbx3 and Cbx5 (17,36). SUV39H2 was reported to be involved in acute stress-induced increase of H3K9 methylation in the hippocampus (37), and also serves a crucial role in DNA repair following double-stranded breakage (18). SUV39H2 is characterized as an embryonic and testis-specific HMT, which is undetectable in other tissues (14). It has been demonstrated that SUV39H2 is upregulated in multiple types of cancer, and serves oncogenic functions in human cancer (18,19); however, there are no selective inhibitors reported for SUV39H2, a potentially important therapeutic target.

The present study examined the expression of various histone methylation modifiers and demonstrated that the expression of SUV39H2 was increased in osteosarcoma cancer cell lines, which is in agreement with the previously reported upregulation of SUV39H2 in osteosarcoma tissue samples (18). However, the expression of SUV39H2 in osteosarcoma tissue samples was not examined in the present study. Reduced expression of SUV39H2 led to a drastic reduction of osteosarcoma cell viability, and overexpression of SUV39H2 promoted cell growth, which indicated that SUV39H2 may serve crucial roles in osteosarcoma development. Furthermore, knockdown of SUV39H2 caused G1 arrest in osteosarcoma cells and induced apoptosis. To further assess the role of SUV39H2 in apoptosis, RNA-seq analysis was performed to identify downstream target genes of. Expression profiling through RNA-seq in HOS cells following SUV39H2 knockdown resulted in 438 DEGs including a set of genes related to the G1/S cell cycle transition that supported these observations. These results indicated the

importance of SUV39H2 in osteosarcoma cells and provided some evidence in support of the hypothesis that SUV39H2 may be a promising therapeutic approach for the treatment of osteosarcoma.

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## Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

## Authors' contributions

LP participated in the whole project and performed most of the work. XY performed most of the additional revised experiments. MZ and XQ participated in the overall design of this study and proposed helpful ideas. XX and RK performed flow cytometry-related experiments. ZL was involved in the conception of the study and also supervised the quality of all the work throughout the entire process. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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