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ORIGINAL ARTICLE

Enhanced Wnt signaling by methylation-mediated loss of SFRP2 promotes osteosarcoma cell invasion

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Abstract Wnt signaling is essential for the initiation and progression of osteosarcoma (OS) tumors and is suppressed by the secreted frizzled-related proteins (SFRPs). The methylation-induced protein degradation reduces the activity of SFRPs and subsequently increases the activity of Wnt signaling. However, whether the methylation of SFRP2, a member of SFRPs, may be involved in the pathogenesis of OS is not known. Here, we investigated the expression levels of SFRP2 in OS specimens. We found that SFRP2 mRNA was significantly decreased and methylation of SFRP2 gene was significantly increased in malignant OS tumors as compared to the paired adjacent non-tumor tissue. Moreover, SFRP2 expression was significantly decreased in the malignant OS cell lines, SAOS2, MG63, and U2OS, but not in the primary osteoblast cells. The demethylation of SFRP2 gene by 5′-aza-deoxycytidine (5-aza-dCyd) in OS cell lines restored SFRP2 expression, at both mRNA and protein levels, and suppressed cell invasion. Furthermore, the demethylation of SFRP2 gene appeared to inhibit nuclear retention of a key Wnt signaling factor, β-catenin, in OS cell lines. Together, these data suggest that SFRP2 may function as an OS invasion suppressor by interfering with Wnt signaling, and the methylation of SFRP2 gene may promote pathogenesis of OS.

Keywords Osteosarcoma (OS) · Secreted frizzled-related proteins (SFRPs) · Methylation · 5-aza-dCyd · Wnt signaling · β-catenin · Cancer invasion

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Introduction

Wnt signaling plays an essential role in a variety of biological processes during development and tissue hemostasis [1, 2]. A delicate control of Wnt signaling is crucial for the proper maintenance of the organism, while aberrant Wnt signaling may lead to developmental defects and disease initiation and progression [1–7]. Osteosarcoma (OS) is a primary malignant bone tumor in humans and is notorious mainly for its distal metastases [8–13]. In the past years, the advances in chemotherapy have assisted general surgery to substantially improve the long-term survival of patients with non-metastatic OS. However, the survival rate for patients with metastatic OS remains low [8–22]. Hence, there is a great need for a better understanding of OS invasion, migration, and metastases.

The frizzled proteins are G-protein-coupled receptors that negatively regulate Wnt signaling [1, 2], which includes canonical and non-canonical pathways. In the canonical Wnt signaling pathway, β-catenin is a key mediator. At the resting state without binding of Wnt ligands to the frizzled/low-density lipoprotein receptor-related protein (LRP) receptor complexes, cytosolic β-catenin forms a multi-protein "destruction complex" with several proteins including adenomatous polyposis coli, Axin, and glycogen synthase kinase-3β (GSK-3β), resulting in the phosphorylation and subsequent degradation of β-catenin. At the activated state, binding of the frizzled/LRP receptor complex with Wnt ligands will alter the composition of the complex, resulting in suppression of β-catenin degradation to induce translocation of β-catenin to the nucleus. Nuclear β-catenin thus interacts with T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to activate target genes [23, 24]. In the non-canonical Wnt pathway which is not solidly defined, β-catenin appears to be dispensable. To summarize, activation of Wnt signaling by certain ligands may activate canonical signaling through

β-catenin, through suppression of β-catenin proteolytic degradation and increase in nuclear translocation and retention [23, 24].

The regulation Wnt signaling is executed by a variety of different modulators including the family of secreted frizzled-related proteins (SFRPs) [25–28]. Till now, five human SFRPs have been detected, and all contain a cysteine-rich domain (CRD) homologous to the frizzled CRD for Wnt ligand binding [25–28]. SFRP proteins have been shown to inhibit activation of canonical Wnt signaling. SFRPs are found to downregulate in several cancers, which are often indicators of poor prognosis [25–28]. Indeed, in recent years, accumulating evidence has supported SFRPs as tumor suppressors, due to their silence in cancer via promoter hypermethylation [25–28]. However, whether the methylation of SFRP2, a member of SFRPs, may be involved in the pathogenesis of OS is not known.

Here, we investigated the expression levels of SFRP2 in OS specimens and malignant OS cell lines, SAOS2, MG63, and U2OS, compared to the primary osteoblast cells. The demethylation of SFRP2 gene was induced by 5′-aza-deoxycytidine (5-aza-dCyd) in OS cell lines, and the effects on levels of SFRP2 expression, suppressed cell invasion, and cellular β-catenin location were analyzed.

Materials and methods

Patient tissue specimens

Twenty-six OS patients (male 12, female 14; aged 28 to 52) were included in the current study. The specimens from these patients were histologically diagnosed at the First Affiliated Hospital of Chongqing Medical University from 2010 to 2014. For the use of these clinical materials for research purposes, prior patient's consents and approval from the Institutional Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University were obtained.

Cell culture of OS cell lines and primary osteoblasts

The malignant OS cell lines U2OS, MG63, and SAOS2 and a primary osteoblast cells (POB) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and grown in 5 % CO_2 at 37 °C in a humidified atmosphere. The culture media were RPMI 1640 medium supplemented with 10 % fetal bovine serum, 105 U/l penicillin, 100 mg/l streptomycin, 2 mmol/l glutamine, 7.5 μg/ml phenol red, 200 nmol/l TPA, 200 pmol/l cholera toxin, 10 nmol/l endothelin-1, and 10 ng/ml human stem cell factor (R&D systems, Minneapolis, MN, USA). As controls, human osteoblasts from a female donor of 54 years of age were isolated from human trabecular bone obtained from the trochanteric region during orthopedic hip replacement surgery. The osteoblasts were cultured in osteoblast growth medium (Invitrogen, CA, Carlsbad, USA) supplemented with 10 % FBS serum with 105 U/l penicillin, 100 mg/l streptomycin, and 2 mmol/l glutamine. The cells were regularly analyzed for the absence of mycoplasma contamination.

Transwell cell invasion assay

The transwell cell invasion assay was performed using a Fluorometric Cell Migration Assay kit with polycarbonate membrane inserts (5-μm pore size; Cell Biolabs, San Diego, CA, USA). The cells were serum-starved overnight in DMEM prior to initiation of the experiment. The cells were then incubated at 37 °C for 24 h to allow cell migration through the membrane. Migratory cells were detached from the underside of the membrane and subsequently lysed and detected by CyQuant GR dye (Invitrogen). Fluorescence measurement was performed in a FluoStar Optima fluorescence plate reader with a 485/520-nm filter set.

RT-qPCR

Total RNA was extracted using RNeasy kit (QIAGEN, Hilden, Germany). For cDNA synthesis, complementary DNA (cDNA) was randomly primed from 2 μg of total RNA using the Omniscript reverse transcription kit (QIAGEN). Real-time quantitative polymerase chain reaction (RT-qPCR) was subsequently performed in triplicate with a 1:4 dilution of cDNA using the Quantitect SyBr green PCR system (QIAGEN). All primers were purchased from QIAGEN. Data were collected and analyzed using $2^{-\Delta\Delta}$Ct method. Values of genes were first normalized against α-tubulin and then compared to the experimental controls.

Western blot

Total protein was extracted and homogenized in RIPA lysis buffer (1 % NP40, 0.1 % SDS, 100 μg/ml phenylmethylsulfonyl fluoride, 0.5 % sodium deoxycholate, in PBS) on ice. The supernatants were collected after centrifugation at 12,000×*g* at 4 °C for 20 min. Protein concentration was determined using a BCA protein assay kit (Bio-Rad, China), and whole lysates were mixed with 4× SDS loading buffer (125 mmol/l Tris-HCl, 4 % SDS, 20 % glycerol, 100 mmol/l DTT, and 0.2 % bromophenol blue) at a ratio of 1:3. Protein samples were heated at 100 °C for 5 min and were separated on SDS-polyacrylamide gels. The separated proteins were then transferred to a PVDF membrane. The membrane blots were first probed with a primary antibody. After incubation with horseradish peroxidase-conjugated second antibody, autoradiograms were prepared using the enhanced chemiluminescent system to visualize the protein antigen. The signals were recorded using X-ray film. Nuclear and cytosol proteins

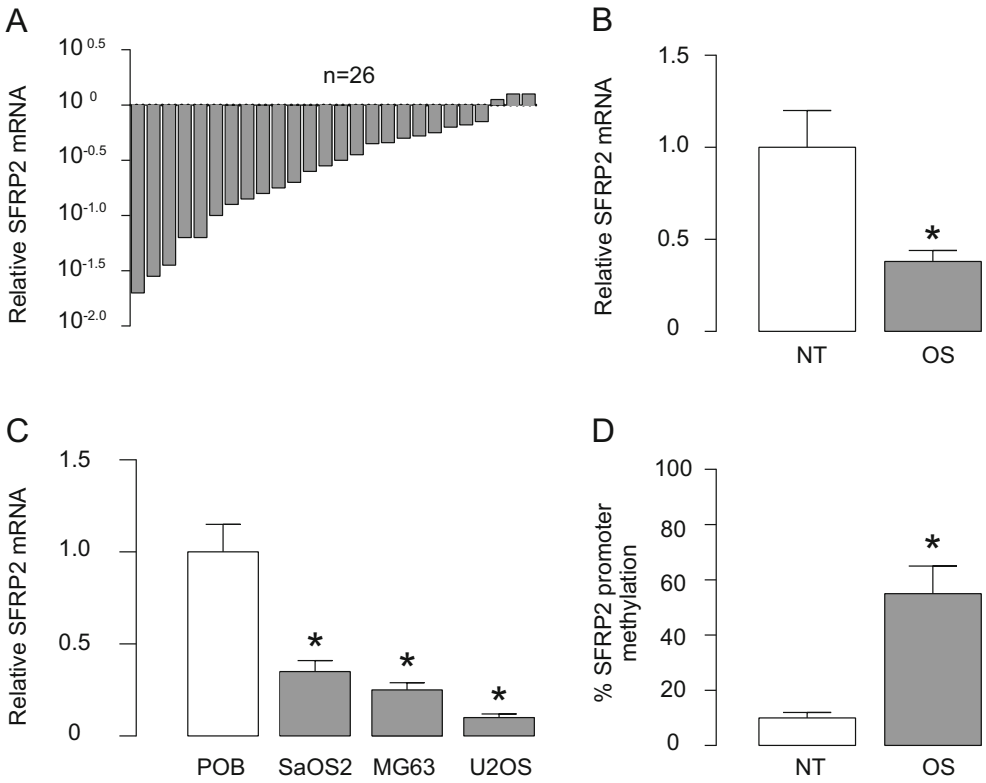

Fig. 1 Decreased SFRP2 expression and increased SFRP2 gene methylation are detected in OS specimens. **a**, **b** SFRP2 mRNA levels were analyzed in malignant OS tumors as compared to the paired adjacent non-tumor tissue (*NT*), shown by individual levels (**a**), and by mean ± SD (**b**). **c** SFRP2 mRNA levels were analyzed in the malignant OS cell lines, SAOS2, MG63, and U2OS, and human osteoblasts (POB). **d** The quantification of SFRP2 gene methylation by BS in specimens. *$p < 0.05$. $N = 30$

were isolated with nuclear and cytoplasmic Extraction Kit (Thermo Scientific, Rockford, IL, USA). Primary antibodies were rabbit anti-SFRP2 (Abcam, Cambridge, MA, USA), anti-β-catenin (Sigma-Aldrich), and anti-α-tubulin (Cell Signaling, San Jose, CA, USA). α-tubulin was used as protein loading controls. Secondary antibody is HRP-conjugated anti-rabbit (Jackson ImmunoResearch Labs, West Grove, PA, USA). Images shown in the figures were representative from five individuals. Densitometry of Western blots was quantified with NIH ImageJ software (Bethesda, MA, USA). The protein levels were first normalized to α-tubulin and then normalized to experimental controls.

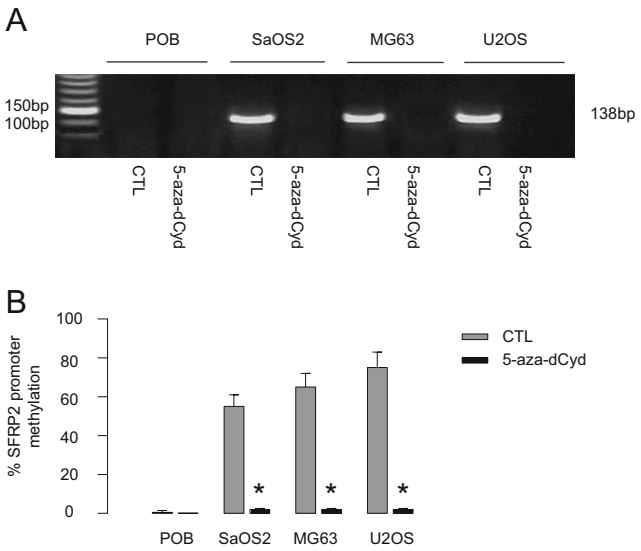

Fig. 2 Demethylation of SFRP2 gene is induced by 5-aza-dCyd in OS cells. We used 5′-aza-deoxycytidine (*5-aza-dCyd*) to treat malignant OS cell lines, SAOS2, MG63, and U2OS, and POB. **a**, **b** The methylation-specific polymerase chain reaction (MSP) was done, shown by representative MSP gels (**a**), and by quantification (**b**). *$p < 0.05$. $N = 5$

MSP analysis

Methylation-specific primers were designed based on the promoter sequence of SFRP2, with 5′-GGGTCGGAGT TTTTCGGAGTTGCGC-3′ as the forward primer and 5′-CCGCTCTCTTCGCTAAATACGACTCG-3′ as the reverse primer, with a PCR product of 138 bp. The sequences 5′-TTTTGGGTTGGAGTTTTTTGGAGTTGTGT-3′ and 5′-AACCCACTCTCTTCACTAAATACAACTCA-3′ were used for the forward and reverse non-methylation-specific primers, respectively, with a PCR product of 145 bp. The following thermal cycling conditions were used: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 10 min. The 138-bp methylation-specific polymerase chain reaction (MSP) product was isolated using electrophoresis in a 1.5 % agarose gel and analyzed using an ultraviolet (UV) gel imaging system (ImageQuant 350; GE Healthcare Co., Little Chalfont, UK).

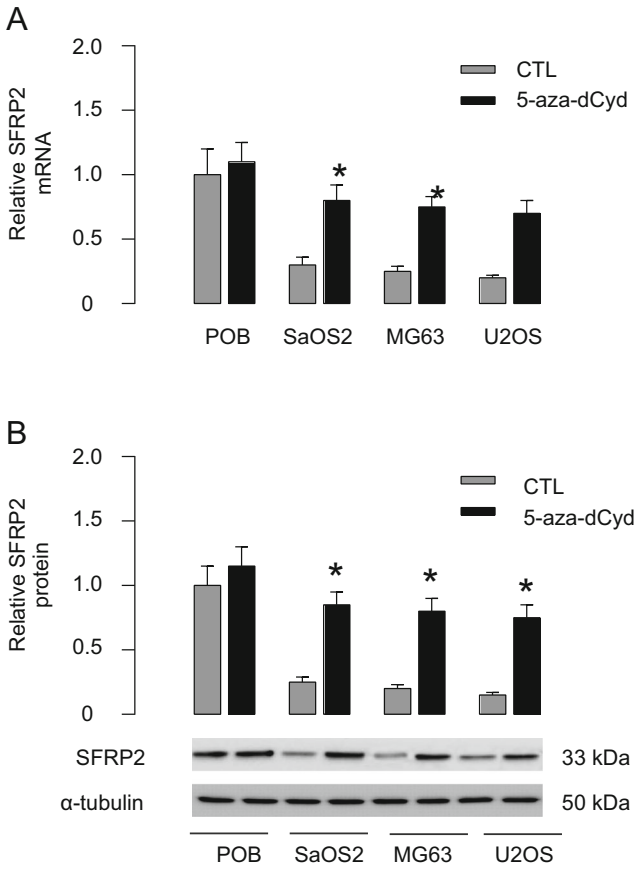

Fig. 3 Demethylation of SFRP2 gene restores SFRP2 expression in OS cells. We used 5′-aza-deoxycytidine (*5-aza-dCyd*) to treat malignant OS cell lines, SAOS2, MG63, and U2OS, and the POB. **a**, **b** The SFRP2 expression was analyzed, at mRNA (**a**) and protein (**b**) levels. $*p < 0.05$. $N = 5$

BS analysis of CTGF promoter methylation

Bisulfite sequencing (BS) primers for the CTGF promoter region were designed to avoid methylated CpGs.

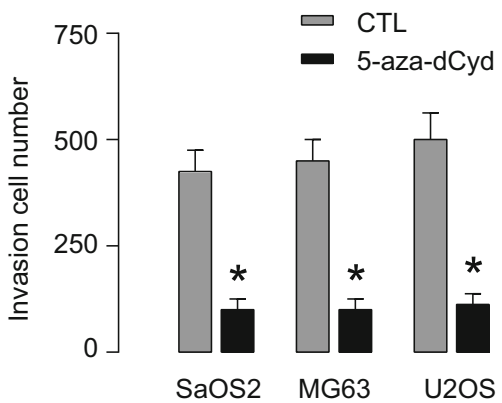

Fig. 4 Demethylation of SFRP2 gene suppresses OS cell invasion. We used 5′-aza-deoxycytidine (*5-aza-dCyd*) to treat malignant OS cell lines, SAOS2, MG63, and U2OS. The cell invasion was analyzed in a transwell cell invasion assay. $*p < 0.05$. $N = 5$

Once the DNA sample treated with sodium bisulfite was fully sulfonated, the BS product was amplified with forward: 5′-TTGTTTGTAAGGTAATTATTAG-3′ and outside reverse 5′-ATTTTCTTAACCTTTTT TATAC-3′ and inside reverse: 5′-AAACAAAAA AAAAACCAAAC-3′, with a product size of about 200 bp. The PCR thermal cycling conditions were initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 45 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s; and final extension at 72 °C for 10 min. The 200-bp amplification product was isolated using electrophoresis in a 1.5 % agarose gel and visualized under UV light. A 10-μl aliquot of the PCR product was subjected to further sequencing by the Beijing Genomics Institute (Beijing, China). The BS primer amplification products from the samples of the three groups were compared with completely sulfonated promoter target sequences using the JellyFish 1.3 data application software (Field Scientific, LCC, Lewisburg, PA, USA). The methylation level was calculated as: $(mC/C - G) \times 100$ %.

Statistics

All statistical analyses were carried out using the GraphPad Prism 6.0 statistical software (GraphPad Software, Inc., La Jolla, CA, USA). All values in cell and animal studies are depicted as mean ± standard deviation and are considered significant if $p < 0.05$. All data were statistically analyzed using one-way ANOVA with a Bonferroni correction, followed by Fisher's exact test for comparison of two groups. Bivariate correlations were calculated by Spearman's rank correlation coefficients.

Results

Decreased SFRP2 expression and increased SFRP2 gene methylation are detected in OS specimens

We investigated the expression levels of SFRP2 in OS specimens. We found that SFRP2 mRNA was significantly decreased in malignant OS tumors as compared to the paired adjacent non-tumor tissue (NT), shown by individual levels (Fig. 1a) and by mean ± SD (Fig. 1b). Moreover, SFRP2 expression was significantly decreased in the malignant OS cell lines, SAOS2, MG63, and U2OS, but not in the POB (Fig. 1c). Next, we checked methylation of SFRP2 gene in malignant OS specimens and found that SFRP2 gene methylation was significantly increased in malignant OS tumors as compared to NT (Fig. 1d).

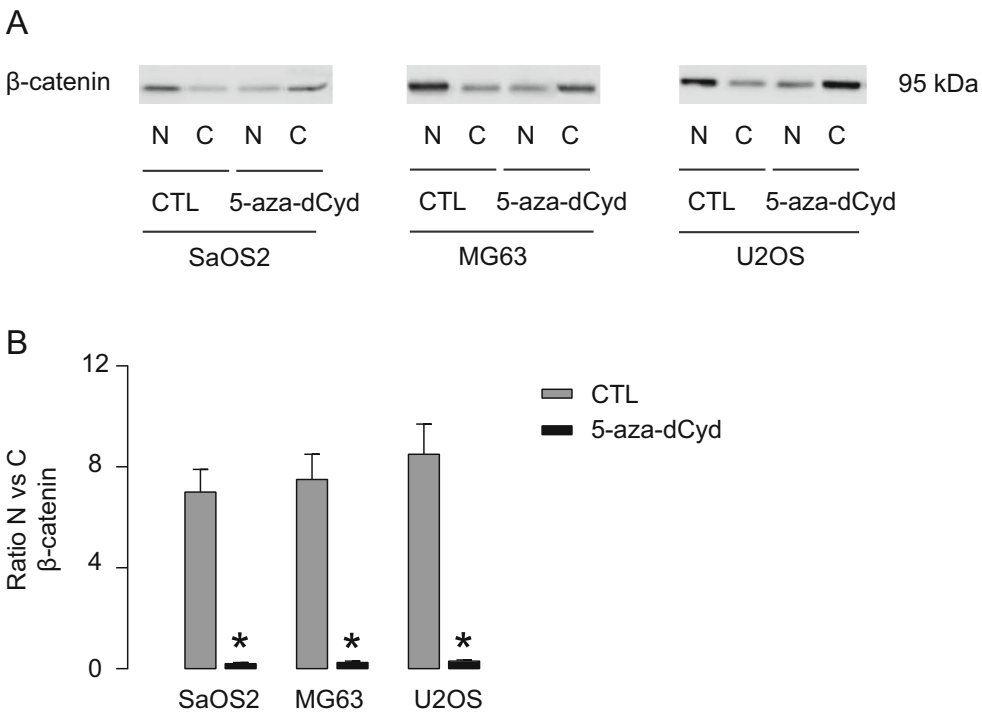

Fig. 5 Demethylation of SFRP2 gene suppresses nuclear retention of β-catenin in OS cells. We analyze the effects of SFRP2 gene demethylation on the activation of canonical Wnt signaling. **a**, **b** The nuclear protein vs. cytosol protein from the 5-aza-dCyd-treated OS cells were analyzed for β-catenin in OS cells SAOS2, MG63, and U2OS, shown by representative blots (**a**) and by quantification of the nuclear vs. cytosol β-catenin ratio (**b**). $*p < 0.05$. $N = 5$

Demethylation of SFRP2 gene is induced by 5-aza-dCyd in OS cells

Then, we used 5-aza-dCyd to treat malignant OS cell lines, SAOS2, MG63, and U2OS, and POB as a control. We found that demethylation of SFRP2 gene is efficiently induced by 5-aza-dCyd in OS cells, shown by representative MSP gels (Fig. 2a), and by quantification (Fig. 2b).

Demethylation of SFRP2 gene restores SFRP2 expression in OS cells

We found that demethylation of SFRP2 gene by 5-aza-dCyd in OS cell lines SAOS2, MG63, and U2OS restored SFRP2 expression, at both mRNA (Fig. 3a) and protein (Fig. 3b) levels. Thus, demethylation of SFRP2 gene restores SFRP2 expression in OS cells.

Demethylation of SFRP2 gene suppresses OS cell invasion

Moreover, in a transwell cell invasion assay, we found that demethylation of SFRP2 gene by 5-aza-dCyd in OS cell lines SAOS2, MG63, and U2OS significantly suppressed cell invasion (Fig. 4). Thus, demethylation of SFRP2 gene suppresses OS cell invasion.

Demethylation of SFRP2 gene suppresses nuclear retention of β-catenin in OS cells

Finally, we aimed to analyze the effects of SFRP2 gene demethylation on the activation of canonical Wnt signaling. Then, we isolated nuclear protein vs. cytosol protein from the 5-aza-dCyd-treated OS cells. We found that demethylation of SFRP2 gene suppressed nuclear retention of β-catenin in OS cells, shown by representative blots (Fig. 5a), and by quantification of the nuclear vs. cytosol β-catenin ratio (Fig. 5b). Together, based on our data in the current study, we proposed that the demethylation of SFRP2 gene may inhibit nuclear retention of the key Wnt signaling factor, β-catenin, in OS cell lines, to facilitate cell invasion. SFRP2 may thus function as an OS invasion suppressor by interfering with Wnt signaling, and the methylation of SFRP2 gene may promote pathogenesis of OS.

Discussion

The SFRP2 plays a pivotal role in the Wnt pathway and mainly functions as an antagonist of activation of Wnt signaling [29, 30]. Previous studies have shown that SFRP2 is a Wnt inhibitor whose promoter CpGs were hypermethylated at high frequency in colorectal cancers (CRCs) [31–37]. Indeed, the methylation of SFRP2 has been mainly investigated in CRCs, and the pattern of SFRP2 methylation appears to differ

throughout the promoter during progressive tumorigenesis, showing that extensive methylation of the SFRP2 promoter was present primarily in CRCs [31–37]. However, a role of SFRP2 gene methylation in the tumorigenesis of OS is ill-defined.

In the current study, we investigated the expression levels of SFRP2 in OS specimens. We found that SFRP2 mRNA was significantly decreased, and this downregulation of SFRP2 may result from the several possible reasons, including promoter methylation. Hence, we analyzed promoter methylation of SFRP2 in patients' specimens, and the results confirmed our hypothesis. Since the SFRP2 expression was also significantly decreased in the malignant OS cell lines, SAOS2, MG63, and U2OS, but not in POB, these data suggest that SFRP2 gene promoter may exist in a relative low methylation state in normal bone tissue, while significantly methylated when the cells undergo phenotypic changes towards malignant OS. Hence, DNA methylation appears to be a regulatory mechanism of SFRP2 expression, possibly contributing to the regulation of Wnt signaling, and the pathogenesis of OS. Understanding the role of the demethylation of the SFRP2 promoter in the development of OS may lead to the identification of novel strategies and/or additional therapeutic targets for the prevention and treatment of malignant OS.

Based on these clinical findings, we were prompted to analyze the effects of demethylation of SFRP2 on the tumor cell biology. The demethylation of SFRP2 gene by 5-aza-dCyd in OS cell lines restored SFRP2 expression, at both mRNA and protein levels, resulting in a suppression of cell invasion. We also examined cell survival and growth, using an MTT and CCK-8 assay, but we did not find any alterations of these parameters by SFRP2 gene demethylation. These negative data were not shown in the result part. Thus, our data suggest that the effects of SFRP2 on OS cells may be mainly on cell invasion, rather than cell proliferation and survival.

Furthermore, the demethylation of SFRP2 gene appeared to inhibit nuclear retention of a key Wnt signaling factor, β-catenin, in OS cell lines. Hence, the regulation of SFRP2 gene methylation directly regulated canonical pathway of Wnt signaling. Together with a pivotal role of Wnt signaling in the tumorigenesis of OS, our study suggests that SFRP2 may function as an OS invasion suppressor by interfering with Wnt signaling, and the methylation of SFRP2 gene may promote pathogenesis of OS.

The present study had several limitations. The current molecular mechanism analyses were performed only in vitro. The analyses on an animal model may further provide evidence of a role of SFRP2 promoter methylation in the carcinogenesis of OS. In addition, despite the fact that previous studies and the current work have indicated that methylation is involved in the regulation of SFRP2 expression in malignant OS cells, the precise mechanisms underlying the control of SFRP2 gene promoter methylation still requires further investigation.

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Compliance with ethical standards

Conflicts of interest The authors have declared that no competing interests exist.

References

1. Nojima M, Suzuki H, Toyota M, Watanabe Y, Maruyama R, Sasaki S. Frequent epigenetic inactivation of sfrp genes and constitutive activation of Wnt signaling in gastric cancer. Oncogene. 2007;26:4699–713.
2. Suzuki H, Watkins DN, Jair KW, Schuebel KE, Markowitz SD, Chen WD, et al. Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. Nat Genet. 2004;36:417–22.
3. Lin R, Feng J, Dong S, Pan R, Zhuang H, Ding Z. Regulation of autophagy of prostate cancer cells by beta-catenin signaling. Cell Physiol Biochem. 2015;35:926–32.
4. Xu X, Ma J, Li C, Zhao W, Xu Y. Regulation of chondrosarcoma invasion by MMP26. Tumour Biol. 2015;36:365–9.
5. Liu S, Chen M, Li P, Wu Y, Chang C, Qiu Y, et al. Ginsenoside rh2 inhibits cancer stem-like cells in skin squamous cell carcinoma. Cell Physiol Biochem. 2015;36:499–508.
6. Ge Z, Zhang B, Bu X, Wang Y, Xiang L, Tan J. Molecular mechanism of activating protein-4 regulated growth of hepatocellular carcinoma. Tumour Biol. 2014;35:12441–7.
7. Zhang H, Liu C, Kong Y, Huang H, Wang C, Zhang H. Tgfbeta signaling in pancreatic ductal adenocarcinoma. Tumour Biol. 2015;36:1613–8.
8. Tsuchiya H, Tomita K, Mori Y, Asada N, Morinaga T, Kitano S, et al. Caffeine-assisted chemotherapy and minimized tumor excision for nonmetastatic osteosarcoma. Anticancer Res. 1998;18:657–66.
9. Yang J, Zhang W. New molecular insights into osteosarcoma targeted therapy. Curr Opin Oncol. 2013;25:398–406.
10. Li G, Fu D, Liang W, Fan L, Chen K, Shan L, et al. Cyc1 silencing sensitizes osteosarcoma cells to trail-induced apoptosis. Cell Physiol Biochem. 2014;34:2070–80.
11. Liu Y, He J, Chen X, Li J, Shen M, Yu W, et al. The proapoptotic effect of formononetin in human osteosarcoma cells: involvement of inactivation of ERK and Akt pathways. Cell Physiol Biochem. 2014;34:637–45.
12. Wang Q, Cai J, Wang J, Xiong C, Zhao J. MiR-143 inhibits EGFR-signaling-dependent osteosarcoma invasion. Tumour Biol. 2014;35:12743–8.
13. Xiao Q, Zhang X, Wu Y, Yang Y. Inhibition of macrophage polarization prohibits growth of human osteosarcoma. Tumour Biol. 2014;35:7611–6.
14. Luo XJ, Tang DG, Gao TL, Zhang YL, Wang M, Quan ZX, et al. MicroRNA-212 inhibits osteosarcoma cells proliferation and invasion by down-regulation of Sox4. Cell Physiol Biochem. 2014;34:2180–8.
15. Li F, Li S, Cheng T. Tgf-beta1 promotes osteosarcoma cell migration and invasion through the miR-143-versican pathway. Cell Physiol Biochem. 2014;34:2169–79.
16. He Y, Meng C, Shao Z, Wang H, Yang S. MiR-23a functions as a tumor suppressor in osteosarcoma. Cell Physiol Biochem. 2014;34:1485–96.

17. Chen J, Fu H, Wang Z, Yin F, Li J, Hua Y, et al. A new synthetic ursolic acid derivative IUA with anti-tumor efficacy against osteosarcoma cells via inhibition of JNK signaling pathway. Cell Physiol Biochem. 2014;34:724–33.
18. Xu H, Liu X, Zhao J. Down-regulation of miR-3928 promoted osteosarcoma growth. Cell Physiol Biochem. 2014;33:1547–56.
19. Xu G, Wang J, Jia Y, Shen F, Han W, Kang Y. Mir-142-3p functions as a potential tumor suppressor in human osteosarcoma by targeting HMGA1. Cell Physiol Biochem. 2014;33:1329–39.
20. Pan W, Wang H, Jianwei R, Ye Z. MicroRNA-27a promotes proliferation, migration and invasion by targeting MAP2k4 in human osteosarcoma cells. Cell Physiol Biochem. 2014;33:402–12.
21. Chen X, Luther G, Zhang W, Nan G, Wagner ER, Liao Z, et al. The E-F hand calcium-binding protein S100A4 regulates the proliferation, survival and differentiation potential of human osteosarcoma cells. Cell Physiol Biochem. 2013;32:1083–96.
22. Chang YW, Zhao YF, Cao YL, Gu XF, Li ZQ, Wang SQ, et al. Liver x receptor alpha inhibits osteosarcoma cell proliferation through up-regulation of FoxO1. Cell Physiol Biochem. 2013;32:180–6.
23. Pinto D, Clevers H. Wnt, stem cells and cancer in the intestine. Biol Cell. 2005;97:185–96.
24. Holland JD, Klaus A, Garratt AN, Birchmeier W. Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol. 2013;25:254–64.
25. Ehrlund A, Mejhert N, Lorente-Cebrian S, Astrom G, Dahlman I, Laurencikiene J, et al. Characterization of the Wnt inhibitors secreted frizzled-related proteins (SFRPS) in human adipose tissue. J Clin Endocrinol Metab. 2013;98:E503–508.
26. Shin H, Kim JH, Lee YS, Lee YC. Change in gene expression profiles of secreted frizzled-related proteins (SFRPS) by sodium butyrate in gastric cancers: induction of promoter demethylation and histone modification causing inhibition of Wnt signaling. Int J Oncol. 2012;40:1533–42.
27. Nathan E, Tzahor E. sFRPS: a declaration of (Wnt) independence. Nat Cell Biol. 2009;11:13.
28. Drescher U. A no-Wnt situation: SFRPS as axon guidance molecules. Nat Neurosci. 2005;8:1281–2.
29. Zhang Y, Chen H. Genistein attenuates WNT signaling by up-regulating sFRP2 in a human colon cancer cell line. Exp Biol Med (Maywood). 2011;236:714–22.
30. Chung MT, Lai HC, Sytwu HK, Yan MD, Shih YL, Chang CC, et al. SFRP1 and SFRP2 suppress the transformation and invasion abilities of cervical cancer cells through Wnt signal pathway. Gynecol Oncol. 2009;112:646–53.
31. Sui C, Ma J, Chen Q, Yang Y. The variation trends of SFRP2 methylation of tissue, feces, and blood detection in colorectal cancer development. Eur J Cancer Prev. 2015. doi: 10.1097/CEJ.0000000000000185
32. Zhang X, Song YF, Lu HN, Wang DP, Zhang XS, Huang SL, et al. Combined detection of plasma GATA5 and SFRP2 methylation is a valid noninvasive biomarker for colorectal cancer and adenomas. World J Gastroenterol. 2015;21:2629–37.
33. Sui C, Wang G, Chen Q, Ma J. Variation risks of SFRP2 hypermethylation between precancerous disease and colorectal cancer. Tumour Biol. 2014;35:10457–65.
34. Takeda M, Nagasaka T, Dong-Sheng S, Nishie H, Oka T, Yamada E, et al. Expansion of CpG methylation in the SFRP2 promoter region during colorectal tumorigenesis. Acta Med Okayama. 2011;65:169–77.
35. Pehlivan S, Artac M, Sever T, Bozcuk H, Kilincarslan C, Pehlivan M. Gene methylation of SFRP2, P16, DAPK1, HIC1, and MGMT and KRAS mutations in sporadic colorectal cancer. Cancer Genet Cytogenet. 2010;201:128–32.
36. Wang DR, Tang D. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. World J Gastroenterol. 2008;14:524–31.
37. Huang Z, Li L, Wang J. Hypermethylation of SFRP2 as a potential marker for stool-based detection of colorectal cancer and precancerous lesions. Dig Dis Sci. 2007;52:2287–91.