Histone Methyltransferase WHSC1 Inhibits Colorectal Cancer Cell Apoptosis via Targeting Anti-apoptotic BCL2

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Abstract

Background: WHSC1 is a histone methyltransferase that facilitates histone H3 lysine 36 dimethylation (H3K36me2), which is a permissive mark associated with active transcription. Colorectal cancer (CRC) is the 4th deadliest and 3rd most frequent cancer globally. However, the role of WHSC1 in CRC progression remains unknown.

Methods: qRT-PCR, immunoblotting assays (WB), and immunohistochemistry (IHC) staining was performed to investigate WHSC1 expression levels in CRC tissues and normal tissues. CCK-8 assays, colony formation assays, and flow cytometry were also used to assess the effect of WHSC1 depletion in CRC cell proliferation, apoptosis and oxaliplatin sensitivity in vitro. A cell line-derived xenograft model in nude mice was performed to determine the role of WHSC1 in CRC cell apoptosis in vivo.

Results: WHSC1 as well as H3K36me2 were highly expressed in clinical CRC tissues compared with in normal counterparts. High WHSC1 expression was correlated with poorer prognosis in CRC patients. Knockdown of WHSC1 significantly promoted CRC cell apoptosis and inhibited tumour growth in vivo. Further mechanistic investigation revealed that WHSC1 directly binds to the promoter region of BCL2 gene and regulate its H3K36 dimethylation level, so BCL2 expression is markedly decreased after WHSC1 depletion.

Conclusions: Our findings demonstrated that knockdown of WHSC1 promoted colon cancer cell apoptosis and suppressed CRC tumorigenesis through targeting BCL2 transcription, suggesting WHSC1 activity may be a potential therapeutic target for the treatment of CRC.

Background

Colorectal cancer (CRC) is the 4th deadliest and 3rd most frequent cancer globally[1]. Within colorectal cancer cells, BCL2 is well known to act to suppress the induction of apoptosis[2]. In the colon cancer patient tissue BCL2 is often abnormally highly expressed, and higher frequencies of BCL2 positive cells are linked with lower overall rates of apoptosis[3]. It is therefore vital that we understand how cancer cells modulate BCL2 expression to subvert apoptosis, in order to develop new treatments for this deadly disease.

Traditionally, epigenetic regulation refers to diverse and reversible chemical modifications on DNA or histones. Among these diverse modifications, histone methylation at different sites lead to increased or inhibited gene expression, which regulate gene expression in a way independent to genome changes[4, 5]. Epigenetic regulation is widely reported to participate in the development and progression of colon cancer[6], and histone methyltransferases are key players in this regulation. Take for instance, enhancer of zeste homolog 2 (EZH2), which is linked to colorectal cancer tumorigenesis[7, 8]. The histone methyltransferase WHSC1 facilitates dimethylation of lysine 36 on the H3 histone (H3K36me2), which activates transcription at modified loci[9]. In the published results, WHSC1 knockout mice showed cardiac developmental defects[10], and WHSC1 is necessary for B cell development[11]. In addition, WHSC1 also
has been shown to play important roles in cancer, it is highly expressed in many human cancers and affects the cell cycle by regulating the WNT signaling pathway[12]. WHSC1 also directly interacts with NF-κB to activate additional target genes that have been linked with improved survival of prostate cancer cells[13]. In prostate cancer, WHSC1 control cancer metastasis by targeting transcription factor TWIST1 and mTORC2 signaling[14, 15].

Here in our study, we firstly reported that WHSC1 and H3K36me2 levels were elevated in human CRC, and WHSC1 inhibited colon cancer cell apoptosis by directly binding to the promoter region of BCL2. WHSC1 depletion inhibited BCL2 expression and suppressed CRC tumorigenesis both in *vitro* and *in vivo*. WHSC1 reduction promotes the Oxaliplatin sensitivity in CRC cells. Our findings thus demonstrate that WHSC1 activity may be a potential therapeutic target in colon cancer.

**Materials And Methods**

**Human specimen analysis.** Twenty-three colon cancer and paracancerous specimens were taken from Shanghai Ninth People's Hospital. The study was approved by by Research Ethics Committee of Shanghai Ninth People's Hospital.

**RNA Extraction and Real-Time Polymerase Chain Reaction.** The RNAiso Plus reagent (Takara) was used for RNA extraction, and a PrimeScript RT Kit (Takara) was used for reverse transcription or 1.5μg RNA based on provided protocols. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCRs) was conducted with the 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA). GAPDH was used as a normalization control. Primers sequence were listed in Table S1.

**Western Blot.** Loading lysis buffer was used to extract total protein from cells. The Lowry protein assay was then used for protein level quantification. 30mg of protein lysate were then run on an SDS/PAGE gel and transferred to a PVDF membrane (Millipore, Milford, MA), probed with appropriate primary and HRP–conjugated secondary antibodies in a 5% bovine serum albumin solution. Antibodies used were: H3K36me2 (Abways, AY2513), WHSC1 (ActiveMotif, 39880), BCL2 (Abways, AY1364), GAPDH (ABways, AB0036), Cleaved caspase 3 (Cell Signaling Technology, 9664), Ki67(Cell Signaling Technology, 9449).

**CCK-8 Assay.** The Cell Counting Kit-8 (Beyotime) was used in accordance with provided protocols. Briefly, 2000 cells/well were added to a 96-well plate, 10ul CCK-8 solution was added, cells were incubated for 2 hours, and absorbance was then read.

**Colony formation assay.** 1000 cells were plate per well in 6-well plates. After 10 days, Crystal Violet Staining Solution (Beyotime, C0121) was used to stain cells and all colonies with 20 or more cells were counted.

**Flow Cytometry.** $1 \times 10^5$stable WHSC1 konck-down and control cells were plated in a 6 well plate. 48 hours later, cells were collected for Flow Cytometry analysis. For cell cycle analysis using a Cell Light Edu Kit (Ruibo, C10313). 7AAD and Annexin-V-PE (BD Biosciences) were used for apoptosis staining.
**Tumorigenesis Assay.** $2 \times 10^6$ HCT116 cells that had been transduced with appropriate lentivirus were injected subcutaneously in a total volume of 200μL into the right flank of nude mice. Every 3 days, tumor size was measured. The formula used to assess tumor volume was $V = \frac{1}{2}(a*b*b)$, with $a$ being the major and $b$ the minor tumor axes. Mice were euthanized after 3-4 weeks, and tumor weight was assessed. Experiment were consistent with the European Community guidelines for the use of experimental animals.

**Cell Lines, Plasmids, Reagents, and Cell Transduction.** The colon cancer cell lines HCT15, SW480, HCT116, COLO205, and DLD-1 were bought from the American Type Culture Collection. The normal NCM460 epithelial line was bought from Gaining Biotechnology. DMEM supplemented with 10% fetal calf serum was used to grow all cells. The FuGENE® HD Transfection Reagent (Promega) was used for all transfections in accordance with manufacturer's instructions. For WHSC1 knockdown, a WHSC1-specific shRNA was cloned into the pLVX-shRNA1 plasmid (Clontech). WHSC1-shRNA1: GTGCCAATAACACGTCCACT, WHSC1-shRNA2: GCCCTTCGCAGTGTTTGTCT. For a negative control, a scrambled sequence was used. Packaging was conducted with a three plasmid-system with pSPAX2 and pMD2G. Lentiviral supernatant was used to infect cell lines, which were then selected for two weeks with 2mg/mL puromycin.

**ChIP-qPCR.** $1 \times 10^6$ CRC cells were crosslinked and lysed. UCD-300 (Bioruptor) was used to shear crosslinked DNA to ~200-1000 base pairs in length. ChIP was performed according to manufacturer's instructions of Chromatin Immunoprecipitation Kit (17–371; Millipore), followed by qPCR for quantification of ChIP-enriched DNA. The antibodies used for ChIP were anti-H3K36me3 (Active Motif; 61021) and normal mouse IgG (Active Motif; 12-371B). Primers sequence were listed in Table S2.

**Statistical analysis**

All of the results were repeated at least three times except when indicated otherwise. Data were presented as mean ± SEM. 2-tailed paired Student's t-test was applied to compare the differences between two groups. Log-rank tests were used to calculate the Kaplan–Meier survival curve comparison. $p<0.05$ was considered statistically significant.

**Results**

**Elevated WHSC1 expression in human CRC.**

In order to determine the role of WHSC1 in colorectal cancer, we initially investigated WHSC1 expression in CRC patient by quantitative reverse-transcriptase PCR (RT-qPCR). WHSC1 expression was elevated significantly in cancerous tissues relative to normal counterparts (Fig. 1A). Next, Western blots showed that WHSC1 protein levels were both increased in these CRC tumors relative to normal controls (Figure 1B). Simultaneously, we used immunohistochemical detection of a colorectal cancer tissue microarray to analyze WHSC1 expression in 97 archived metastasis-free human CRC specimens by immunohistochemistry (IHC). Our results demonstrated that patients with high WHSC1 expression level
showed shorter metastasis-free survival (MFS) times than patients with low levels of WHSC1, which indicated that WHSC1 might be associated with the risk of CRC progression and could be an independent predictor of MFS (Fig. 1C). In addition, H3K36me2 levels were significantly elevated in CRC tumor tissue compared with normal counterparts as assessed by immunohistochemistry (Fig. 1D). Together, these data suggested WHSC1 as a potential CRC biomarker, and a possible causal role of WHSC1 in the development of CRC.

**WHSC1 reduction inhibits tumorigenesis in CRC cells.**

To assess WHSC1 function in CRC, we knocked down WHSC1 in two CRC cell lines, HCT116 and DLD1, using pLVX-shRNA1 plasmid with control shRNA and two independent WHSC1 shRNA constructs. Both mRNA and protein levels of WHSC1 were confirmed to be significantly decreased after shRNA transduction in both two cell lines (Fig. 2A and B). Reduced WHSC1 significantly inhibited tumor cell growth ability as assessed by CCK-8 and colony formation assay (Figure 2C and D). These data suggested that WHSC1 benefited CRC progression in vitro.

**WHSC1 ablation increases CRC cell apoptosis in vitro.**

Apoptosis and cell cycle are two important factors that relative to CRC progression. We therefore assessed cell apoptosis by Annexin V-7AAD assay and cell cycle progression by Edu assay in WHSC1-knockdown (WHSC1-KD) colon cancer cells. Data revealed that cell cycle progression seemed to be intact after knocking down WHSC1 (Figure 3A and B). But WHSC1 knockdown significantly increased apoptosis in HCT116 and DLD1 cell (Figure3C and D). The above data indicated that the inhibited cell viability in WHSC1-deficient colon cancer cells were due to increased cell apoptosis rather than decreased cell proliferation.

**WHSC1 depletion inhibits CRC development in vivo by promoting apoptosis.**

We next used mouse xenograft models to investigate the importance of WHSC1 in CRC development in vivo. HCT116 cells were transfected with WHSC1 or control shRNA and stable cell lines were established by puromycin screening. Cells were the subcutaneously implanted into left armpit of nude mice. Tumor volumes were monitored, and mice were euthanized at 3 weeks post-implant to measure tumor weight. We found that WHSC1 knock-down lead to delayed tumor progression and decreased tumor weight as compared with control CRC cells (Figure 4A-C). Immunohistochemistry assay revealed that there were more TUNEL-positive and caspase-positive cells in WHSC1-knockdown tumor group compared with control group (Figure4D and E). However, there were no significant difference between two groups in ki67-positive cells (Figure4D and E). These results indicated that WHSC1 promotes tumor development by inhibiting apoptosis in vivo.

**WHSC1 promotes anti-apoptotic BCL2 expression.**

In order to explore the mechanism how WHSC1 inhibits apoptosis in CRC, we checked mRNA and protein level of apoptosis-related genes. We found that the BCL2 mRNA and protein level were markedly
decreased after WHSC1 depletion (Figure 5A). However, other genes associated with apoptosis, such as BAD, BAX and BIRC5, were intact in WHSC1-KD cells (Figure 5A). Protein level of BCL2 was also significantly reduced in WHSC1-KD HCT116 and DLD1 cell lines (Figure 5B). Consistently, there were also significantly decreased BCL2 expression level in WHSC1-knockdown xenografted tumor tissue compared with control xenografted tumor as assessed by immunohistochemistry and western blot (Figure 5C and D). These findings suggest that WHSC1 might mediate cell apoptosis of CRC by controlling BCL2 expression.

**WHSC1 mediates H3K36me2 modification at transcriptional initiation region of BCL2.**

Since WHSC1 is well known for its histone methyltransferase activity, and H3K36me2 is associated with active gene transcription[9]. We hypothesized that if WHSC1 bind to transcriptional initiation region of BCL2 gene and directly induced H3K36 dimethylation so as to promote BCL2 transcription. Chromatin immunoprecipitation (ChIP) was performed in CRC cells to assess this possibility. WHSC1 was significantly enriched at 1 to 1.5 (P3) kilobases and 2 to 2.5 kilobases (P5) upstream of the transcription start site (TSS) (Figure 5E). And levels of H3K36me2 modification at the loci of P3 and P5 in WHSC1-knockdown CRC cells were significantly lower than that in control cells (Figure 5E). Therefore, our results indicated that BCL2 is a direct target gene of WHSC1, WHSC1 promoting BCL2 transcription by controlling the H3K36 dimerization modification in its transcription initiation region, so as to regulate colon cancer cell apoptosis.

**WHSC1 reduction promotes oxaliplatin sensitivity of CRC cells.**

In addition to cancer cell apoptosis, BCL2 was also reported to confer chemoresistance in colorectal cancer[16]. What’s more, BCL2 Protein Family were used to establish a model to predict responses to chemotherapy in colorectal cancer[17]. Oxaliplatin is commonly used as standard first line treatment for colon cancer[18]. We further investigate if WHSC1 and H3K36me modification play a role in oxaliplatin resistance. WHSC1-KD and control CRC cells were treated with different concentrations of oxaliplatin. Results revealed that oxaliplatin-induced apoptosis was markedly higher in WHSC1-KD cells compared with in control cells (Figure 6A). In addition, after Oxaliplatin treatment, the clonogenic capacity of WHSC1-KD cells also decreased significantly (Figure 6B). Therefore, we summarized that inhibition of WHSC1 expression increases the oxaliplatin sensitivity of colon cancer cells.

**Discussion**

BCL2 is highly expressed in many colon cancer patients to inhibit apoptosis[2, 3]. Therefore, the specific targeting of BCL2 to promote apoptosis may be a viable therapeutic approach. Here we show that the methyltransferase WHSC1 is also highly expressed in colon cancer samples, and WHSC1 bind to BCL2 gene promoter, changing the H3K36me2 modification state to increase its transcription. WHSC1 depletion reduces BCL2 expression, leading to elevated cell apoptosis during CRC progression. Overall, this study elucidates a new epigenetic regulation mechanism during CRC progression and suggests that inhibiting
WHSC1 enzymatic activity and H3K36 methylation state to promote colon cancer cell apoptosis, might be a new approach to therapeutic CRC treatment to be achieved.

So far, WHSC1 importance in colon cancer progression is rarely understood. BCL2 is one of the most known anti-apoptotic factors and found to be highly expressed in a variety of solid tumors relating to tumorigenesis[19–21]. So our results suggest a close relationship between the epigenetic regulator, WHSC1, and key intracellular oncogenes. WHSC1 knockdown significantly decreased the H3K36me2 modification level in BCL2 gene locus, leading to inhibition of transcription of this key oncogene. We believe that the regulation of BCL2 gene expression was an important but may not all effect of WHSC1 in tumorigenesis of CRC. WHSC1 has previously been linked to transcription initiation[9], but whether WHSC1 also regulates other important genes associated with colon cancer necessitates ChIP-seq to verify.

As we have established the oncogenic role of WHSC1 in CRC cell apoptosis, we didn’t determine which oncogenic insults led to elevated WHSC1 expression in CRC cells. In a previous study, it was reported that increased AKT activity directly phosphorylates WHSC1 at S172 to promote its stability, preventing WHSC1 degradation, and lead to prostate cancer metastasis[15]. Previous evidence has indicated that the PI3K/Akt signal pathway activates signals for cell survival and cell proliferation in CRC[22]. AKT activation was inhibited by lipid phosphatase activity of a major tumor suppressor PTEN (phosphate and tension homology deleted on chromosome ten) and played an important role in carcinogenesis of CRC[23]. In our study, it was found that PTEN expression was significantly downregulated in colon cancer tissues (data not shown), so we suspected that this may be a cause of elevated WHSC1 expression in colon cancer cells.

In addition to transcriptional regulation, histone methyltransferases may also modify non-histone molecules. Histone methyltransferase enhancer of zeste homolog 2 (EZH2) was found to be able to catalyze K49 dimethylation of STAT3[24], which is crucial for the expression of many IL-6–dependent genes. SET domain–containing protein 6 (SETD6) was identified as a protein lysine methyltransferases (PKMT) that monomethylated RelA at Lys310, which rendered RelA inert and attenuated RelA-driven transcriptional programs, including inflammatory responses in primary immune cells[25]. Whether WHSC1 can act similarly on non-histone proteins remains to be established.

Conclusions

In summary, our findings demonstrate that WHSC1 is highly expressed in CRC tissues, and WHSC1 expression is closely related to prognosis of CRC. WHSC1 depletion significantly promotes colon cancer cell apoptosis and suppressed CRC tumorigenesis. WHSC1 regulates BCL2 expression by directly binds to the promoter region of BCL2 gene and regulate its H3K36 dimethylation level. These findings provide a new mechanism in CRC tumorigenesis and suggest WHSC1 activity may be a potential therapeutic target for the treatment of CRC.
Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all patients, and the study protocol was approved by the Ethics Committee of Shanghai Ninth People's Hospital. All animal research was carried out following approval by the Institutional Animal Care and Use Committee of Shanghai Ninth People's Hospital.

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YW, XT and XJM designed and performed the experiments, MG and GS assisted with IHC and WB experiments, KZ and DCC analysed and interpreted data, WJP and YCH discussed the paper and provided useful suggestions. LDH and LMZ contributed to the study conception and design. YW and XJM drafted the manuscript. All authors have read and approved the final manuscript.

References

1. Fearon ER. Molecular Genetics of Colorectal Cancer. Annu Rev Pathol-Mech. 2011;6:479-+.
2. Baretton GB, Diebold J, Christoforis G, Vogt M, Muller C, Dopfer K, Schneiderbanger K, Schmidt M, Lohrs U. Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas - Aspects of carcinogenesis and prognostic significance. Cancer. 1996;77(2):255–64.
3. Jiang M, Milner J. Bcl-2 constitutively suppresses p53-dependent apoptosis in colorectal cancer cells. Brit J Cancer. 2003;88:34–4.
4. Tran TQ, Lowman XH, Kong M. Molecular Pathways: Metabolic Control of Histone Methylation and Gene Expression in Cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2017;23(15):4004–9.
5. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nature reviews Cancer. 2011;11(10):726–34.

6. Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. Annu Rev Genom Hum G. 2002;3:101–28.

7. Wang CG, Ye YJ, Yuan J, Liu FF, Zhang H, Wang S. EZH2 and STAT6 expression profiles are correlated with colorectal cancer stage and prognosis. World J Gastroentero. 2010;16(19):2421–7.

8. Kodach LL, Jacobs RJ, Heijmans J, van Noesel CJM, Langers AMJ, Verspaget HW, Hommes DW, Offerhaus GJA, van den Brink GR, Hardwick JCH. The role of EZH2 and DNA methylation in the silencing of the tumour suppressor RUNX3 in colorectal cancer. Carcinogenesis. 2010;31(9):1567–75.

9. Li Y, Trojer P, Xu CF, Cheung P, Kuo A, Drury WJ, Qiao Q, Neubert TA, Xu RM, Gozani O, et al. The Target of the NSD Family of Histone Lysine Methyltransferases Depends on the Nature of the Substrate. J Biol Chem. 2009;284(49):34283–95.

10. Nimura K, Ura K, Shiratori H, Ikawa M, Okabe M, Schwartz RJ, Kaneda Y. A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf-Hirschhorn syndrome. Nature. 2009;460(7252):287-U157.

11. Campos-Sanchez E, Deleyto-Seldas N, Dominguez V, Carrillo-De-Santa-Pau E, Ura K, Rocha PP, Kim J, Aljoufi A, Esteve-Codina A, Dabad M, et al. Wolf-Hirschhorn Syndrome Candidate 1 Is Necessary for Correct Hematopoietic and B Cell Development. Cell Rep. 2017;19(8):1586–601.

12. Toyokawa G, Cho HS, Masuda K, Yamane Y, Yoshimatsu M, Hayami S, Takawa M, Iwai Y, Daigo Y, Tsuchiya E, et al. Histone Lysine Methyltransferase Wolf-Hirschhorn Syndrome Candidate 1 Is Involved in Human Carcinogenesis through Regulation of the Wnt Pathway. Neoplasia. 2011;13(10):887-U826.

13. Yang P, Guo L, Duan ZJ, Tepper CG, Xue L, Chen X, Kung HJ, Gao AC, Zou JX, Chen HW. Histone methyltransferase NSD2/MMSET mediates constitutive NF-kappaB signaling for cancer cell proliferation, survival, and tumor growth via a feed-forward loop. Molecular cellular biology. 2012;32(15):3121–31.

14. Ezponda T, Popovic R, Shah MY, Martinez-Garcia E, Zheng Y, Min DJ, Will C, Neri A, Kelleher NL, Yu J, et al. The histone methyltransferase MMSET/WHSC1 activates TWIST1 to promote an epithelial-mesenchymal transition and invasive properties of prostate cancer. Oncogene. 2013;32(23):2882–90.

15. Li N, Xue W, Yuan H, Dong B, Ding Y, Liu Y, Jiang M, Kan S, Sun T, Ren J, et al. AKT-mediated stabilization of histone methyltransferase WHSC1 promotes prostate cancer metastasis. J Clin Investig. 2017;127(4):1284–302.

16. Wu DW, Huang CC, Chang SW, Chen TH, Lee H. Bcl-2 stabilization by paxillin confers 5-fluorouracil resistance in colorectal cancer. Cell death differentiation. 2015;22(5):779–89.

17. Lindner AU, Concannon CG, Boukes GJ, Cannon MD, Llambi F, Ryan D, Boland K, Kehoe J, McNamara DA, Murray F, et al. Systems analysis of BCL2 protein family interactions establishes a model to predict responses to chemotherapy. Cancer research. 2013;73(2):519–28.
18. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham C, Zaninelli M, Clingan P, Bridgewater J, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. New Engl J Med. 2004;350(23):2343–51.

19. Reed JC. Bcl-2 on the brink of breakthroughs in cancer treatment. Cell death differentiation. 2018;25(1):3–6.

20. Timucin AC, Basaga H, Kutuk O. Selective targeting of antiapoptotic BCL-2 proteins in cancer. Medicinal research reviews. 2019;39(1):146–75.

21. Smerage JB, Budd GT, Doyle GV, Brown M, Paoletti C, Muniz M, Miller MC, Repollet MI, Chianese DA, Connelly MC, et al. Monitoring apoptosis and Bcl-2 on circulating tumor cells in patients with metastatic breast cancer. Molecular oncology. 2013;7(3):680–92.

22. Huang XF, Chen JZ. Obesity, the PI3K/Akt signal pathway and colon cancer. Obesity reviews: an official journal of the International Association for the Study of Obesity. 2009;10(6):610–6.

23. Jhawer M, Goel S, Wilson AJ, Montagna C, Ling YH, Byun DS, Nasser S, Arango D, Shin J, Klampfer L, et al. PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer research. 2008;68(6):1953–61.

24. Dasgupta M, Dermawan JK, Willard B, Stark GR. STAT3-driven transcription depends upon the dimethylation of K49 by EZH2. Proc Natl Acad Sci USA. 2015;112(13):3985–90.

25. Levy D, Kuo AJ, Chang Y, Schaefer U, Kitson C, Cheung P, Espejo A, Zee BM, Liu CL, Tangsombatvisit S, et al. Lysine methylation of the NF-kappaB subunit RelA by SETD6 couples activity of the histone methyltransferase GLP at chromatin to tonic repression of NF-kappaB signaling. Nature immunology. 2011;12(1):29–36.

Figures
Figure 1

Elevated WHSC1 expression in human CRC. (A) WHSC1 mRNA levels in 23 matched tumor and paracarcinoma tissues (paired t-test, P < 0.001). (B) Immunoblotting analysis of WHSC1 expression in four matched CRC tissues (T) and adjacent noncancerous tissues (N). (C) Kaplan–Meier plot of overall survival of CRC patients based on WHSC1 levels. A log-rank test was used for statistical analysis. (D) Expression of H3K36m2 was assessed by immunohistochemistry. H3K36m2 staining indexes using a 10-point quantification are shown.
Figure 2

Knock down WHSC1 inhibits tumorigenesis in CRC cells. (A) qPCR analysis of the indicated mRNA in control and WHSC1 knockdown cell lines. (B) Immunoblotting analysis of WHSC1 in DLD-1 and HCT116 cells transduced with two specific shRNA. (C and D) CCK-8 assays (C) and colony formation assays (D) in parental and WHSC1 knock down cells. The quantitation results are shown in the right panels.
Figure 3

WHSC1 ablation promotes CRC cell apoptosis. (A) Profiling of the cell cycle distribution of HCT116 and DLD1 cells transiently expressing shCon, shWHSC1-1 or shWHSC1-2. (B) Quantitative analyses of the cell cycle distribution as described in A, assessed by two-sided Student's t-test. (C and D) Flow cytometry profiling and quantification of Annexin-V/7-AAD-stained cells transiently expressing shCon, shWHSC1-1 or shWHSC-2. All data represent the mean±S.D. of three independent experiments. *P<0.05, **P<0.01.
Figure 4

WHSC1 reduction suppresses CRC cell growth and promotes apoptosis in vivo. (A) Tumor growth in mice injected with Hct116 cells stably expressing shWHSC1-1 or the control. (B-C) Tumor volume (B) and volume weight (C) in mice injected with HCT116 cells stably expressing control or shWHSC1-1 (n = 6). (D and E) Immunohistochemistry results for TUNEL and caspase3 assays of tumors derived from HCT116 cells stably expressing control or shWHSC1-1. *P<0.05, **P<0.01.
Figure 5

WHSC1 promotes BCL2 transcription through upregulating H3K36me2 modification on the gene loci. (A-B) The mRNA of apoptosis related genes in the WHSC1-knockdown HCT116 cells compared with control cells. (B) The BCL2 protein level in the WHSC1 knock down Hct116 and DLD1 cells compared with control cells. (C and D) Immunohistochemical and Western detection of BCL2 expression WHSC1 knock down xenografted tumor tissue compared with control tissue. (E) ChIP-qPCR analysis of the binding of H3K36me2 to the BCL2 promoter in HCT116 cells. (F) ChIP-qPCR analysis for anti-H3K36me2 along with control IgG at the BCL2 promoter loci in control or WHSC1 knock down HCT116 cells.
Figure 6

WHSC1 reduction promotes the Oxaliplatin sensitivity in colon cancer cells. (A) shWHSC1 and control CRC cells were treated with oxaliplatin for 48 hours as indicated. The percentage of cells entering apoptosis was determined by flow cytometry using FITC-labeled Annexin V staining. (B) The HCT116 stable-transfected cells were exposed to oxaliplatin for 7 days, and colony formation assays were performed. *P<0.05, **P<0.01.