**Molecular and Cellular Pathobiology**

**hSETD1A Regulates Wnt Target Genes and Controls Tumor Growth of Colorectal Cancer Cells**

Tal Salz¹, Guangyao Li², Frederic Kaye³,⁵, Lei Zhou²,⁵, Yi Qiu⁴,⁵, and Suming Huang¹,⁵

**Abstract**

hSETD1A is a member of the trithorax (TrxG) family of histone methyltransferases (HMT) that methylate H3K4 at promoters of active genes. Although misregulation of mixed lineage leukemia (MLL) family proteins has been associated with acute leukemia, the role of hSETD1A in cancer remains unknown. In this study, we report that hSETD1A and its associated H3K4me3 are upregulated in human colorectal cancer cells and patient samples. Depletion of hSETD1A inhibits colorectal cancer cell growth, colony formation, and tumor engraftment. Genome-wide expression profiling of colorectal cancer cells reveals that approximately 50% of Wnt/β-catenin target genes are affected by the hSETD1A knockdown. We further demonstrate that hSETD1A is recruited to promoters of those Wnt signaling target genes through its interaction with β-catenin, a master regulator of the Wnt signaling pathway. The recruitment of the hSETD1A HMT complex confers promoter-associated H3K4me3 that leads to assembly of transcription preinitiation complex and transcriptional activation. Furthermore, the expression levels of hSETD1A are positively correlated with H3K4me3 enrichment at the promoters of Wnt/β-catenin target genes and the aberrant activation of these genes in human colorectal cancer. These results provide new biologic and mechanistic insights into the cooperative role of hSETD1A and β-catenin in regulation of Wnt target genes as well as in colorectal cancer cell growth in vitro and in vivo. Cancer Res; 74(3); 775–86. ©2013 AACR.

**Introduction**

The mammalian hSETD1A gene belongs to the trithorax (TrxG) family of histone methyltransferases (HMT) that methylates lysine 4 at histone H3 tails (H3K4; 1–4). In mammals, there are six hSET1/mixed lineage leukemia (MLL) complexes. Each complex contains a distinct conserved SET domain containing enzymatic subunit (hSETD1A, hSETD1B, MLL1, MLL2, MLL3, or MLL4). In addition, the hSET1/MLL complexes comprise of several integrated subunits, WDR5, RBBP5, ASH2L, and HCF1, that are required for enzymatic activity (3, 4). Together, they shape the genomic landscape of H3K4 methylations as well as determine normal gene expression patterns and cell identity. Trimethylation of H3K4 (H3K4me3) is highly enriched at transcription start sites (TSS) of active genes and controls gene transcription (1, 3–5). Interestingly, it was recently shown that ablation of hSETD1A, but not MLLs, exhibits the most dramatic effects on global H3K4me3 enrichment and gene expression (6, 7).

Alterations in histone modifications can drive important cancerous processes, such as proliferation, invasion, angiogenesis, and differentiation, by perturbing normal gene expression patterns (8, 9). It has been previously shown that altered H3K4me3 levels are associated with colorectal cancer (10, 11). In addition, global H3K4me3 is dramatically increased during epithelial–mesenchymal transition, a process characterized by the loss of cell adhesion and increased cell mobility (12–14). In contrast, the active removal of H3K4 methylation is important for the opposite process, mesenchymal–epithelial transition, and is partially mediated by LSD1 and JARID1B histone H3K4 demethylases (15, 16). These findings suggest an important role of H3K4me3 in cancer development, maintenance, and progression. However, it remains unknown which HMT is responsible for the increase in H3K4me3 levels in colorectal cancer and what are the target genes affected by the altered H3K4me3 levels during carcinogenesis. Although the effect of TrxG HMTs on gene expression is thought to be global, recent studies revealed that these enzymes interact with tissue-specific transcription factors, suggesting that hSETD1/MLL complexes might regulate a specific developmental pathway in a cell context–dependent manner (17). Therefore, understanding of target gene specificity of the hSET1/MLL...
complexes in specific types of cancers such as colorectal cancer will not only provide new insight into pathogenesis of colorectal cancer, but also lead to new strategies for cancer diagnosis and therapeutic approaches.

hSETD1A has been shown to play a role in cell differentiation and tissue development (4), but very little is known about the role of hSETD1A in cancer, particularly in solid tumors. Interestingly, it was recently shown that hSETD1A can interact with β-catenin (18), a master regulator of the canonical Wnt signaling pathway (19–21), in human embryonic kidney 293 (HEK293) cells. Activation of this pathway results in stabilization and accumulation of β-catenin in the nucleus where it interacts with T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors to activate Wnt target gene transcription (19–21). Such transcription program stimulates cancer-related processes, such as cellular proliferation, invasion, migration, and angiogenesis (19–22). The Wnt signaling pathway also plays an important role in tissue maintenance and regeneration by regulating self-renewal and differentiation of intestinal stem cells (ISC) at the intestinal crypt (22–25). Disturbance of these normal developmental processes often leads to intestinal malignancies (22). Approximately 70% to 90% of all colorectal cancers are driven by the perturbed Wnt signaling pathway (26, 27). Most interestingly, various missense mutations within the hSETD1A gene were found in cancer specimens, including colorectal cancers, suggesting that hSETD1A is perhaps targeted during carcinogenesis (http://www.sanger.ac.uk/genetics/CGP/cosmic/). Although the function of these mutations in colorectal cancer is unknown, the mutants render the intact catalytic SET domain, suggesting that these mutants may remain enzymatically active in cancer.

Here, we investigate the biologic and mechanistic role of hSETD1A in human colorectal cancer cells and patients. We show that hSETD1A and its associated H3K4me3 levels are upregulated in colorectal tumors and that hSETD1A affects cellular growth of colorectal cancer cells as well as primary mouse xenograft tumors. We further demonstrate that hSETD1A regulates the expression levels of a subset of Wnt/β-catenin target genes through its interactions with β-catenin. Importantly, hSETD1A levels in colorectal tumors are positively correlated with H3K4me3 occupancy at promoters of those Wnt/β-catenin target genes. Thus, our data suggest that hSETD1A-mediated H3K4me3 regulates Wnt target genes in colorectal cancers.

Materials and Methods

Cell lines and gene knockdown
Colorectal cancer cell lines were maintained in Dulbecco’s Modified Eagle Medium supplemented with 10% FBS and 1% penicillin–streptomycin (Invitrogen). RKO and HT29 cells were maintained in Minimum Essential Medium or RPMI plus 10% FBS, respectively. FHs int-74 cells, kindly provided by Dr. M. Zajac-Kaye (University of Florida, Gainesville, FL), were in addition supplemented with 10 μg/mL insulin and 30 ng/mL EGF. All colorectal cancer cell lines were obtained from American type culture Collection (ATCC), authenticated, and maintain in early passages, no more than 6 months after receipt from ATCC. Lentiviral TRC vector harboring shRNAs targeting SETD1A gene (Clone ID: TRCN0000152242) or CTNNB1 gene (TRCN0000003846) were obtained from the University of Florida Health Cancer Center shRNA library (Thermo Scientific). pSuper-shSETD1A was described previously (28). Infectious viruses were generated and infected cells as previously described (28). All shRNA sequences were listed in Supplementary Table S4.

Coimmunoprecipitation
Nuclear extract (0.7 mg/IP) was dialyzed overnight, precleared with 5 μg of rabbit immunoglobulin G (IgG) for 2 hours, and then immunoprecipitated with antibodies against β-catenin (C2206) or components of the hSETD1A complex. The precipitated proteins were washed, fractionated in SDS PAGE, and analyzed by Western blot analysis as previously described (28).

Immunohistochemistry and immunofluorescence
Tissues were fixed by formalin and subsequently paraffin embedded. Immunohistochemistry and immunofluorescence were carried out by the Molecular Pathology and Immunology Core at the University of Florida.

Chromatin immunoprecipitation
Cells were cross-linked with 1% formaldehyde plus 1.5 mmol/L ethylene glycol bis(succinimidylsuccinate) at room temperature. Tissue samples were sliced using razor blades followed by formaldehyde cross-linking. Tissue samples were then homogenized, lysed, and filtered. Cross-linked chromatin was sonicated and precipitated with antibodies against specific histone modifications or transcription factors. The precipitated DNA was quantitated by quantitative PCR. The relative fold enrichment was determined by the following equation: 2(Ct(IP)–Ct(ref)). The chromatin immunoprecipitation (ChIP) primers are listed in Supplementary Table S2.

Reverse transcription PCR
Total RNA was isolated using the RNA isolation kit according to the manufacturer’s instructions (Qiagen). RNA (2 μg) was reverse transcribed using the Superscript II Reverse Transcriptase (Invitrogen). cDNA was analyzed using a quantitative PCR with a CFX real-time PCR detection system (Bio-Rad). The relative RNA levels of different Wnt target genes were measured by normalizing with GAPDH, which were further normalized to the Scramble shRNA control. Primer sequences are listed in Supplementary Table S3.

Cell proliferation and colony formation assays
Cells were seeded in 10 cm culture dishes at a density of 2 × 10^4 cells per plate and the cell number was determined by cell viability count. Colony formation assays were performed by seeding cells in 6 cm culture dishes at a density of 1,000 cells per plate. Colonies were stained with crystal violet and counted. Soft agar colony formation assays were performed by seeding 1 × 10^3 cells in culture media containing 0.7% agarose on top of a base layer containing 0.5% agarose. Colonies (>50 cells) were counted after 3 weeks.
Microarray analysis
Ten micrograms of RNA was reverse transcribed to cDNA using the Applied Biosystems High Capacity cDNA Reverse Transcription Kit according to the manufacturer’s instructions (Applied Biosystems). cDNA products were treated with 100 ng RNaseA and then purified using the Qiagen PCR purification kit according to the manufacturer’s instructions. NimbleGen Human Gene Expression array was purchased from Roche Applied Sciences. cDNA samples were labeled, Cy3 hybridized, and processed at the FSU NimbleGen Microarray Facility at Florida State University. The well-characterized Wnt target genes were selected for analysis according to the Stanford University Wnt Home Page (http://www.stanford.edu/group/nusselab/cgi-bin/wnt/) and from the commercially available SABiosciences Wnt-Signaling Targets PCR array. The expression data were analyzed using the bioconductor packages Oligo (29) and Limma (30). Expression was background-corrected and quantile-normalized with RMA (robust multi-array analysis, and the empirical Bayes approach in Limma package corrected for multiple testing.

Colorectal cancer patient samples
Colon adenoma-carcinoma and adjacent normal mucosa tissue were obtained from the Cooperative Human Tissue Network at the University of Alabama at Birmingham (IRB No. 031078 and 010294 University of Alabama). All samples were deidentified. The use of all human tissues for this study was approved by the Institutional Review Board (IRB-01) of the University of Florida (Non-Human Exempt IRB approval No. 24-2012). All procedures were performed according to the regulations and guidelines of the approved protocol.

Mouse xenograft models
The Scramble control or shSETD1A (C7) expressed HCT116 cells (1 × 10^6) mixed with Matrigel in 1:1 ratio with 1 × PBS were injected subcutaneously into the flank of 5- to 6-week-old athymic nude mice. Tumors were harvested 16 days after injection and the volume of each tumor was determined using the formula L × 2 × W × (π/6), L = shortest diameter, W = longest diameter. Mice were purchased from Harlan Labs and housed in the Cancer and Genetics Research Center at the University of Florida (Gainesville, FL). All experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of the University of Florida.

Results
hSETD1A is upregulated in human colorectal cancer
It has been shown that genome-wide enhancer/promoter H3K4 methylation profiles in colorectal cancers are significantly altered compared to the paired normal mucosa (10, 11). The data suggest that specific H3K4 methyltransferases may play an important role in colorectal tumor development. To gain insights into the role of TrxG HMTs in colorectal cancer, we first examined the expression levels of hSETD1A and MLL1 in various human colorectal cancer cell lines. Although MLL1 levels remained unchanged, hSETD1A levels markedly increased in all colorectal cancer cell lines tested comparing to the human normal intestinal cell line FHs Int-74 (Fig. 1A). Moreover, the upregulation of hSETD1A in these colorectal cancer cells was accompanied by a global elevation in H3K4me3 levels (Fig. 1A). Furthermore, the mRNA levels of hSETD1A and hSETD1B were also increased in human colorectal cancer specimens compared with adjacent paired normal mucosa collected from 24 patients (Fig. 1B and Supplementary Fig. S1A). More than 62% of human colorectal cancer specimens showed a more than 2-fold increase in hSETD1A transcript levels (15/24 patients; Fig. 1B), whereas only 26% of patients showed a more than 2-fold elevation in hSETD1B mRNA levels (6/23 patients; Supplementary Fig. S1A). Immunohistochemical staining further confirmed that hSETD1A was aberrantly expressed in colorectal cancer tissues in comparison with adjacent normal mucosa (Fig. 1C).

Importantly, hSETD1A expression was also positively correlated with a global increase in H3K4me3 levels in these colorectal cancer specimens (Fig. 1C). The distribution pattern of hSETD1A in paired normal mucosa showed that hSETD1A was highly expressed at the mouse and human intestinal crypt bottom, but gradually decreased as cells moving toward top of the crypts (Fig. 1D and Supplementary Fig. S1B). The intestinal crypt bottom is populated with highly proliferative and poorly differentiated ISCs while the top of crypt is occupied by differentiated enterocytes (24). Several studies argue that early molecular changes in the crypt bottom drive the initiation of colorectal polyps (22). The fact that hSETD1A levels are elevated in human colorectal cancer and in intestinal crypts implies that hSETD1A activity may be required for intestinal cell proliferation.

hSETD1A plays an important role in colorectal cancer cell proliferation
Next, we determined the effect of hSETD1A on the cellular proliferation of HCT116 colorectal carcinoma cells. hSETD1A was silenced in HCT116 cells using retrovirus harboring shRNA specifically against the hSETD1A gene (Fig. 2A). Knockdown of hSETD1A led to a specific decrease in global H3K4me3 modification in the pooled hSETD1A knockdown cells, as well as decreases in two individual knockdown clones, C7 and C19 (Fig. 2A). In contrast, it did not affect other histone modifications, including H3K4me1, H3K4me2, H3K27me3, and H3K9me2 (Fig. 2A and Supplementary Fig. S2A). The cell viability assay revealed that hSETD1A knockdown significantly inhibited HCT116 cell proliferation (Fig 2B). Furthermore, hSETD1A ablation in several other colorectal cancer cell lines, including SW48, C2A, and RKO, also led to a decrease in the cellular proliferation, similar to that of HCT116 cells (Fig. 2C and Supplementary Fig. S2B and S2C). The hSETD1A silenced HCT116 and SW48 cells also displayed smaller colonies compared with the Scramble control in the colony forming assay (Fig. 2D and E). When grown on soft agar, the shSETD1A expressed HCT116 cells exhibited fewer and smaller colonies with approximately...
45% less colonies in the hSETD1A knockdown cell pool, and average 70% less colonies in individual hSETD1A knockdown clones compared with the Scr control (Fig. 2F).

Furthermore, we did not observe any sub-G1 phase population nor did we observe increased cell death by using fluorescence-activated cells sorting analysis or Trypan blue exclusion dye, respectively, indicating that there is not a significant induction of apoptosis in hSETD1A knockdown cells (Supplementary Fig. S3A and S3B). However, hSETD1A knockdown cells were reduced in the S-phase of the cell cycle (Supplementary Fig. S3A). Thus, we conclude that hSETD1A and its associated H3K4me3 play an important role in regulating the proliferation of colorectal cancer cells, perhaps by controlling a particular oncogenic transcription program.

hSETD1A regulates Wnt target gene transcription

To gain mechanistic insights into the role of hSETD1A in colorectal cancer, we carried out a genome-wide microarray analysis in the Scr control and hSETD1A knockdown HCT116 cells. A total of 6,286 genes were differentially expressed (cutoff \( P \) value of 0.005) in the hSETD1A knockdown cells compared with the Scr control cells (Fig. 3A). Among them, 2,863 genes were upregulated, whereas 3,423 genes were downregulated upon hSETD1A loss (Fig. 3A). Interestingly, almost 1% of the total differentially expressed genes are Wnt signaling target genes (56/6,286; Fig. 3A), representing approximately 50% (56/113) of the total known Wnt target genes. Further analysis revealed that the probability of these Wnt targets being affected by the hSETD1A knockdown was significantly higher than that of the random distribution of genome-wide hSETD1A-affected genes (FDR adjusted \( P \) value \( = 4.67E-8 \); Fig. 3B; Supplementary Table S1).

Key Wnt signaling target genes involved in proliferation, invasion, migration, and angiogenesis were downregulated in the hSETD1A-depleted cells (31/56), whereas target genes repressed by this pathway, such as E-cadherin (CDH1) and SOX9, were upregulated (Fig. 3C; Supplementary Table S1). Upregulation of other Wnt target genes could be an indirect effect of hSETD1A knockdown. The effects of hSETD1A silencing on the expression of selected Wnt target genes and two unaffected genes, CLDN7 and CCNA2, were further validated by quantitative reverse transcription (qRT)-PCR in HCT116 and SW48 cells (Fig. 3C and D). We also confirmed that the knockdown of hSETD1A in HCT116 cells reduced the protein levels of three important Wnt target genes; c-Myc (MYC),...
Snail (SNAI1), and TCF4 (TCF7L2; Fig. 4A). Thus, our data indicate that hSETD1A regulates a subset of Wnt signaling target genes in colorectal cancer cells.

hSETD1A interacts with β-catenin and mediates H3K4me3 at TSS of Wnt target genes

To elucidate the mechanism by which hSETD1A controls Wnt target gene transcription, we examined the effects of hSETD1A knockdown on the expression levels of β-catenin in HCT116 and SW48 cells. The knockdown of hSETD1A in HCT116 cells did not affect miRNA expression of β-catenin (CTNNB1; Supplementary Table S1). Similarly, the protein levels of β-catenin in HCT116 and SW48 cells were unaffected by hSETD1A knockdown (Figs. 2C and 4A). These results suggest that hSETD1A does not regulate β-catenin transcription or its protein stability. Interestingly, recent studies demonstrated that hSETD1A can interact with β-catenin in human embryonic kidney 293 (HEK293) cells (18). To test whether hSETD1A collaborates with β-catenin in regulating Wnt target genes in colorectal cancers, HCT116 or SW48 nuclear extracts were precipitated with antibodies against β-catenin or components of the hSETD1A complex followed by immunoblotting with antibodies against hSETD1A or β-catenin, respectively. As expected, endogenous β-catenin interacted with hSETD1A and components of the hSETD1A complex in both HCT116 and SW48 colorectal cancer cells (Fig. 4B and C). Consistent with the role of the Wnt signaling pathway in maintaining and regulating intestinal crypts and ISC self-renewal (22–25), immunofluorescence staining of β-catenin in colorectal tumor tissue and paired normal mucosa revealed that β-catenin is...
highly expressed in the normal crypt bottom as well as in colorectal tumor tissues (Fig. 4D) where hSETD1A is also highly expressed (Fig. 1C). Thus, β-catenin and hSETD1A might collaborate to regulate the expression of Wnt target genes.

Next, we asked whether hSETD1A and β-catenin complexes are both recruited to promoters of the Wnt/β-catenin target genes. To test this possibility, we performed ChIP assays using antibodies against hSETD1A, RbBP5, Ash2L, WDR5, as well as β-catenin complex, including β-catenin and TCF4. Compared with the IgG control, both hSETD1A and β-catenin complexes were simultaneously bound to the known β-catenin/TCF-responsive elements at the MYC and VEGFA genes in HCT116 cells (Fig. 4E and Supplementary Fig. S3C). Moreover, the knockdown of hSETD1A in HCT116 and SW48 cells led to a significant decrease in H3K4me3 enrichment at the TSSs of several Wnt/β-catenin target genes involved in cell proliferation, invasion, migration, and angiogenesis, such as MYC, SNAIL, VEGFA, MMP7, and TWIST2 (Fig. 4F and Supplementary Fig. S4A and S4B). In contrast, we did not observe any significant change in H3K4me3 levels at the TSS of CDH1 (Fig. 4F and Supplementary Fig. S4A and S4B). Furthermore, β-catenin binding to its target genes was unaffected by hSETD1A knockdown in HCT116 cells (Fig. 4G). These results suggest that recruitment of the hSETD1A complex and subsequent trimethylation of H3K4 at proximal promoters of the Wnt/β-catenin target genes are required for β-catenin action.
Recruitment of hSETD1A by β-catenin is critical for β-catenin–mediated transcriptional activation

We next sought to further examine whether hSETD1A is recruited by β-catenin to the Wnt target gene promoters. To test this possibility, β-catenin was silenced in HCT116 cells using two individual lentiviral shRNAs (Fig. 5A). As expected, the depletion of β-catenin led to a decrease in the expression of its target genes (Fig. 5B) and inhibited cellular proliferation (Supplementary Fig. S4C). Interestingly, H3K4me3 levels were also significantly decreased at the TSSs of the Wnt/β-catenin target genes upon the loss of β-catenin in HCT116 cells (Fig. 5C and Supplementary...
These results indicate that recruitment of hSETD1A at the proximal promoters of Wnt/β-catenin target genes is independent on hSETD1A (Fig. 4G). hSETD1A occupancy at the promoters is dependent on PHD domain containing TAF3 to H3K4me3 mark at active promoters of the selected Wnt/β-catenin target genes. The recruitment of hSETD1A at the proximal promoters of SNAI1, MYC, and VEGFA was significantly reduced upon β-catenin loss, which is consistent with decreased H3K4me3 enrichment at these loci (Fig. 5C and D). While the recruitment of hSETD1A to the Wnt/β-catenin–responsive promoters is dependent on β-catenin (Fig. 5D), β-catenin occupancy at these promoters is independent on hSETD1A (Fig. 4G). These results indicate that β-catenin recruits hSETD1A to the promoters of the Wnt/β-catenin target genes and relies, at least partially, on hSETD1A-mediated H3K4me3 for transcriptional activation.

A link between promoter associated H3K4me3 and transcriptional activation was proposed to rely on the binding of PHD domain containing TAF3 to H3K4me3 mark at active promoters (31). Therefore, we examined whether binding of TAF3 to the selected Wnt/β-catenin–targeted promoters was impaired by hSETD1A depletion. TAF3 occupancy at the TSSs of MYC, SNAI1, and VEGFA was decreased in the hSETD1A-depleted HCT116 cells as compared with the Scramble control or the negative region (Fig. 5E). Furthermore, we analyzed the overlapping target genes that are corologated by Wnt, TAF3, and hSETD1A in HCT116 cells comparing our microarray data set for hSETD1A knockdown to the TAF3 knockdown HCT116 microarray data set obtained from NCBI GEO2R (31). There are 991 corogulated genes by both TAF3 and hSETD1A [5605 (991) 2735] (Supplementary Fig. S5A), which is highly significant (P = 4.27E-90). Importantly, hSETD1A and TAF3 share 14 Wnt target genes, including MYC, TCF7L2, SNAI2, and MMP7 (Supplementary Fig. S5B).
Thus, β-catenin activates Wnt target genes by recruiting the hSETD1A complex to deposit H3K4me3 marks for transcription preinitiation complex assembly at promoters.

Levels of hSETD1A positively correlate with H3K4me3 enrichment at promoters of Wnt/β-catenin target genes in human colorectal cancer and with sizes of the engrafted tumors

To examine whether hSETD1A levels correlate with H3K4me3 levels at the TSSs of several Wnt/β-catenin target genes in human colorectal cancer tissues, we carried out H3K4me3 ChIP analyses in colorectal tumor tissue and adjacent normal mucosa from patient #1. Both hSETD1A levels and H3K4me3 enrichment at the TSSs of Wnt/β-catenin target genes were upregulated in tumor tissue, as compared with paired normal mucosa from the same patient (Fig. 1B and 6A). Moreover, Wnt/β-catenin target genes were highly expressed in tumors in which hSETD1A levels were highly upregulated (patient #1–3), but not in tumors in which hSETD1A levels were unchanged (patients #7 and #10; Fig. 6B). These results
support our hypothesis that hSETD1A controls promoter H3K4me3 levels, leading to the activation of Wnt/β-catenin target genes and subsequently Wnt pathway–mediated cellular proliferation.

Finally, to assess the role of hSETD1A and its mediated H3K4 trimethylation in colorectal tumor growth, we employed a mouse xenograft model by which the Scramble control and hSETD1A knockdown HCT116 cells were injected subcutaneously into the flank of athymic nude mice, and primary tumor volume was evaluated at 2 weeks postinjection. Consistent with in vitro data, The knockdown of hSETD1A partially suppressed primary tumor growth (Fig. 6C). Given the correlation between hSETD1A levels and colorectal cancer cell growth (Fig 2A, B, and F), we reasoned that the larger tumors that grew from the hSETD1A knockdown cells might result from the reactivation of hSETD1A in mice. To test this possibility, we examined hSETD1A protein levels in different sizes of tumors derived from hSETD1A knockdown HCT116 cells that ranged from small to large (Fig 6D). As expected, the lower hSETD1A levels strongly corresponded to the smaller tumor size and the lower expression levels of c-Myc oncoprotein (Fig. 6D and E). These results suggest that hSETD1A controls colorectal tumor growth in a dose-dependent manner (Fig 2 and 6).

Discussion

Here, we uncover an important pro-proliferative role of hSETD1A in colorectal cancer through its interaction with β-catenin to activate a subset of Wnt target genes. We demonstrate that hSETD1A and its mediated H3K4me3 are aberrantly upregulated in colorectal cancer cell lines and specimens (Fig. 1). Suppression of hSETD1A in several colorectal cancer cell lines leads to a significant decrease in H3K4me3 levels, Wnt target gene expression, and tumor cell growth. H3K4me3 is associated with promoters of active genes and play a critical role in transcriptional activation (1–5). Misregulation of enzymes catalyzing such histone mark may change normal gene expression patterns that control cellular proliferation and differentiation to initiate tumor growth. Thus, these data support that hSETD1A plays an important role in cellular proliferation and tumor growth.

Misregulation of the Wnt signaling pathway is frequently involved in carcinogenesis and leads to 70%–90% of all human colorectal cancer (26, 27), indicating that the Wnt pathway plays a critical role in colorectal cancer. We demonstrate that hSETD1A and β-catenin interact to confer H3K4me3 at the promoters of several Wnt/β-catenin target genes (Fig. 4E and Supplementary Fig. S3D). Loss of β-catenin results in a decrease in hSETD1A occupancy at Wnt-targeted loci. These findings are consistent with previous report that H3K4me3 is reduced at the telomerase (TERT) promoter in the absence of functional β-catenin (18). Together, these data indicate that the hSETD1A HMT complex acts as a coactivator for Wnt target genes in colorectal cancer.

It was previously shown that MLL1 and MLL2 can also interact with β-catenin (32). However, it is unclear whether they play a similar role in regulating Wnt target genes. Epigenomic analysis of H3K4me1 at enhancer elements of genes linked to colorectal cancer indicated that changes in enhancer associated H3K4me1 can also drive a specific transcription program to promote colon carcinogenesis (10). It has been reported that in Drosophila hSETD1A and hSETD1B preferentially catalyze promoter associated H3K4me3 while MLLs (Trx and Trx) are responsible for enhancer-specific H3K4me1 and H3K4me2 (4, 6). Combining with our findings, these data suggest a clever mechanism in which β-catenin activates Wnt target genes by targeting MLL1/2 to confer enhancer-directed H3K4me1 while recruiting hSETD1A to catalyze promoter-associated H3K4me3.

β-Catenin can act as a platform to recruit histone-modifying enzymes such as p300 and MLLs (19, 32, 33). It was further proposed that the β-catenin/TCF/LEF complex mediates the assembly of mediator and TFIID complexes that are required for activation of Wnt target genes (19). However, it is unclear how TFIID is specifically targeted to the promoters of the Wnt target genes. H3K4me3 plays a critical role in transcriptional activation through direct interactions with the PHD domain of TAF3 (31, 34). Our data demonstrated that TAF3 and hSETD1A share a large number of target genes, including 14 Wnt target genes (Supplementary Fig. S4), and hSETD1A-dependent H3K4me3 is critical for the recruitment of TAF3 to Wnt/β-catenin–targeted promoters in colorectal cancer cells (Fig. 5E).

An interesting finding is that hSETD1A is highly expressed and colocalized with β-catenin in the intestinal crypt (Fig. 1D and Supplementary Fig. S1B), where proliferating ISCs reside and the Wnt signaling pathway is highly active (22). The activation of the Wnt signaling pathway is a key event in maintenance of ISC self-renewal and acts as a master switch between the proliferation and differentiation of epithelial cells (22–25). Notably, ectopic activation of Wnt signaling genes in ISCs is capable of initiating intestinal malignancies (22, 25). Thus, it is conceivable that the aberrant activation of hSETD1A in crypt bottoms may cooperate with β-catenin in activating Wnt signaling target genes and initiating oncogenic tumor growth.

On the basis of our results and other studies, we depicted a model demonstrating plausible transcription events that could lead to the development of a colorectal tumor (Fig. 7).

In this model, β-catenin and hSETD1A are upregulated and interact in the nucleus; β-catenin then recruits hSETD1A to putative Wnt promoters to confer H3K4me3, assemble pre-initiation complex, activate Wnt target genes, and subsequently promote cellular growth. Transcriptional activation of Wnt target genes is the last step of the Wnt signaling pathway and is also commonly misregulated step in all colorectal cancers associated with the perturbed Wnt pathway (70%–90% of all colorectal cancers). Identifying and targeting the β-catenin partners or cofactors, such as hSETD1A, could be an effective therapeutic approach for inhibiting the Wnt signaling pathway in colorectal cancer. Future studies investigating the role of hSETD1A in various
Figure 7. A working model illustrating the cooperative role of hSETD1A and β-catenin in regulation of Wnt target genes in colorectal cancer. In normal colon cells, β-catenin levels remain low due to its phosphorylation and subsequent degradation. In colorectal cancer, both hSETD1A and β-catenin levels are upregulated. Localization of β-catenin to the nucleus enables its interactions with hSETD1A HMT. These interactions facilitate promoter-H3K4me3, recruitment of TAF3 (and transcription machinery), activation of Wnt/β-catenin target genes, and cellular growth.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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