DNMT1 and DNMT3B Modulate Distinct Polycomb-Mediated Histone Modifications in Colon Cancer

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Abstract

DNA methylation patterns are established and maintained by three DNA methyltransferases (DNMT): DNMT1, DNMT3A, and DNMT3B. Although essential for development, methylation patterns are frequently disrupted in cancer and contribute directly to carcinogenesis. Recent studies linking polycomb group repression complexes (PRC1 and PRC2) to the DNMTs have begun to shed light on how methylation is targeted. We identified previously a panel of genes regulated by DNMT3B. Here, we compare these with known polycomb group targets to show that ~47% of DNMT3B regulated genes are also bound by PRC1 or PRC2. We chose 44 genes coregulated by DNMT3B and PRC1/PRC2 to test whether these criteria would accurately identify novel targets of epigenetic silencing in colon cancer. Using reverse transcription-PCR, bisulfite genomic sequencing, and pyrosequencing, we show that the majority of these genes are frequently silenced in colorectal cancer cell lines and primary tumors. Some of these, including HAND1, HMX2, and SIX3, repressed cell growth. Finally, we analyzed the histone code, DNMT1, DNMT3B, and PRC2 binding by chromatin immunoprecipitation at epigenetically silenced genes to reveal a novel link between DNMT3B and the mark mediated by PRC1. Taken together, these studies suggest that patterns of epigenetic modifiers and the histone code influence the propensity of a gene to become hypermethylated in cancer and that DNMT3B plays an important role in regulating PRC1 function.

Introduction

DNA methylation is an essential epigenetic mark for mammalian embryonic development and transcriptional regulation. Global patterns of DNA methylation are established and regulated via the action of four DNA methyltransferases (DNMT): DNMT1, DNMT3A, DNMT3B, and DNMT3L (1). DNMT1 associates with S-phase replication foci and acts primarily as a maintenance methyltransferase (2). DNMT3A and DNMT3B are essential for de novo methylation during embryonic development (3). Hypomorphic mutations in the DNMT3B gene result in immunodeficiency, centromere instability, and facial anomalies (ICF) syndrome (3–5). Dnmt3L forms a complex with Dnmt3a and Dnmt3b in embryonic stem cells and stimulates their activity (6, 7).

Aberrant DNA methylation patterns, including CpG island hypermethylation and repetitive region hypomethylation, are hallmarks of transformed cells (8). Misregulation of DNMT expression clearly contributes to tumorigenesis as shown recently by the work of Linhart and colleagues, which showed that elevated Dnmt3b1 expression in the Apc	extsuperscript{Min/+} model enhanced colorectal carcinogenesis and caused tumor suppressor gene methylation (9). DNA methylation, however, does not act alone in repressing gene activity. A complex and intertwined set of post-translational modifications of the core histone tails dynamically imparts both transcriptionally repressive and activating signals. These marks, and the cellular machinery regulating them, are also disrupted in cancer (10). DNMTs, such as DNMT3B, interface directly with the histone code by interacting with the histone methylases SUV39H1 (11) and EZH2 (12), which impart transcriptionally repressive H3K9 and H3K27 methylation marks, respectively.

In mammals, there are two principal polycomb group (PcG) repression complexes: PRC1 and PRC2. PRC2 is composed of EED, EZH2, and SUZ12, whereas PcR1 is composed of HPH1-1, RING1/2, BM11/MEL-18, and HPC1-4. A widely accepted model posits that PRC2 initiates gene silencing by trimming H3K27, followed by recruitment of PRC1 and its associated H2AK119 monoubiquitination activity, resulting in stable maintenance of gene silencing (13). PRC1 and PRC2 are essential for mammalian development and maintenance of embryonic stem cell pluripotency (14). PcG complexes, like the DNMTs, have strong links to cancer. For example, EZH2 is overexpressed in tumors and is predictive of poor prognosis (15). BMI1 cooperates with MYC to promote lymphomas by repressing the INK4a/ARF tumor suppressor locus (16).

Interestingly, connections between DNA methylation and PcG have emerged recently. DNMT1, DNMT3A, and DNMT3B interact with EZH2, and EZH2 targets DNA methylation to certain promoters (12). Schlesinger and colleagues showed that genes subject to tumor-specific hypermethylation in colon cancer were significantly more likely to be marked by H3K27 methylation in normal tissues than genes lacking H3K27 methylation (17). Widschwendter and colleagues showed that PcG targets in embryonic stem cells were 12-fold more likely to sustain cancer-specific hypermethylation (18). Nearly 49% of genes methylated in colon cancer are PcG targets in embryonic stem cells in another recent study (19). In contrast, Gal-Yam and colleagues reported that many genes hypermethylated in a prostate cancer cell line were bound by PcG in normal cells but lost PcG binding upon acquisition of DNA methylation in cancers (20). These studies demonstrate compelling connections between DNA methylation and PcG, yet there is clearly much that we do not understand regarding the molecular mechanisms linking these two systems.

Using cell lines derived from ICF syndrome patients, we recently identified a large number of DNMT3B-regulated genes (21). Whereas the DNA methylation changes in ICF cells were small in many cases,
changes in the histone code were dramatic and this study provided additional support for a link between PcG complexes and DNMT3B in particular (21). In the present article, we have expanded on these studies in the context of colon cancer. We show that there is significant overlap between DNMT3B and PRC1/PRC2 targets. We then asked the question: Can binding of DNMT3B and PRC1 and/or PRC2 predict genes that sustain aberrant DNA hypermethylation in colon cancer? Nearly 80% of 44 randomly chosen genes coregulated by DNMT3B and PRC1 and/or PRC2 were indeed expressed in normal colon but silenced in at least one tumor cell line. Closer examination of 24 of these genes revealed that they were subject to high-frequency epigenetic silencing in colorectal cancer cell lines and primary tumors. Several of these genes, including HAND1, SIX3, HMX2, and TBX4, modulated cell growth in a colony formation assay. Finally, we examined the relationship between DNMT1, DNMT3B, and a large panel of histone marks by chromatin immunoprecipitation. Our analysis revealed an intriguing and hitherto unknown relationship between DNMT3B and the PRC1 mark H2AK119 monoubiquitination, suggesting a functional link between these two important epigenetic modifiers.

Materials and Methods

Cell lines, drug treatments, and human tissues. Human colon tumor cell lines HCT116, HCT15, HT29, SW48, SW480, T84, BKO, and LoVo (American Type Culture Collection) were grown in McCoy's 5A medium (Mediatech). 5-Aza-2′-deoxycytidine (5-aza-dC) was purchased from Sigma. For drug treatments, 5-aza-dC was added to cultures at a final concentration of 1% and cells/tissues were fixed for 10 min. Normal colorectal tissue was cut into small pieces with a scalpel and then transferred to a 35-mm plate with fresh medium containing 800 units/mL (tip model 102, output 5, 50% duty, 12 μm bursts). Fifty micrograms of chromatin were used in each chromatin immunoprecipitation reaction.

Polycomb and DNMT3B Targets in Colon Cancer

Results

Overlap between DNMT3B and PcG target genes. We previously used microarray expression profiling in normal and ICF-derived lymphoblastoid cell lines to identify genes regulated by DNMT3B (21). We further analyzed and refined this data to yield a list of 506 DNMT3B targets. Using data derived from published ChIP-chip and ChIP-seq studies that mapped genome-wide binding of PRC1 and PRC2 subunits and the marks they mediate, we compiled a list of all known PRC1 and/or PRC2 target genes (Supplementary Material). Comparison of these lists revealed that ~47% of DNMT3B target genes were also targets of PRC1 or PRC2, a significant nonrandom enrichment (P < 10−6). Approximately 36% and 35% of DNMT3B target genes were also PRC1 and PRC2 targets, respectively, in embryonic stem cells or embryonic fibroblasts (Fig. 1A).

DNMT3B plus PcG binding in normal cells as a predictor of genes epigenetically silenced in cancer. Recent reports have noted compelling links between genes bound by PcG in normal cells and genes targeted for aberrant DNA methylation in cancer cells (17, 18, 24). For example, Schlesinger and colleagues showed that ∼50% of cancer-methylated genes are premarked by PRC2 and H3K27 methylation in colon cancer and these marks were highly specific because the same genes were not selectively associated with other repressive histone modifications (17). We compiled our own list of genes subject to DNA hypermethylation in human colorectal tumors using recent publications (1,531 genes; Supplementary Material). Comparing our list of genes methylated in colon cancer with the list of PRC1/PRC2 targets we generated revealed that 42% and 44% of cancer-methylated genes are targets of PRC1 and PRC2, respectively, results which are comparable with those of others (17), thus validating our methodology. Use of PRC1 and/or PRC2 binding specifically, or regulation of expression by a DNMT as a predictor of genes undergoing promoter hypermethylation in human tumor cells, however, has not been examined to our knowledge. We therefore performed such an analysis using our data sets, revealing that DNMT3B regulation of gene activity alone (17.2%) is a slightly better predictor of genes methylated in colon cancer than PRC1 only (12.5%; P < 0.01) or PRC2 only (13.5%; P < 0.05). Furthermore, the propensity of a gene for sustaining cancer hypermethylation was very significantly different between the targets of PRC2 combined with DNMT3B (25.6%) compared with PRC2 only (13.5%; P < 0.01). Interestingly, genes regulated by DNMT3B and marked by PRC1 (28.9%; P < 10−7) or by both PRC1 and PRC2 (30.8%; P < 10−9) were the most prone to cancer-specific DNA hypermethylation (Fig. 1A).

Our in silico analyses revealed that DNMT3B regulation and PRC1/PRC2 binding were good predictors of genes susceptible to acquiring aberrant DNA hypermethylation in cancer. To validate these predictions experimentally and determine if these criteria could identify novel cancer methylation targets, we randomly chose 44 genes from the region of overlap between DNMT3B and PRC1 or PRC2 (Fig. 1A, 236 genes) and examined their expression in normal colon and the HCT116 colon cancer cell line. Among the 44 genes selected from the region of overlap with DNMT3B, 37 were PRC1
targets, 35 were PRC2 targets, and 28 were marked by both PRC1 and PRC2 (Fig. 1A). Using semiquantitative RT-PCR, we determined that expression of approximately 13%, 48%, and 39% of the genes was absent, low, and moderate, respectively, in normal colon (Fig. 1B). These results were reproducible in two other normal colon samples (data not shown). To determine whether these genes are also subject to epigenetic silencing in colon cancer and examine the role of DNMT1 and DNMT3B in this process, we monitored their expression in parental HCT116 (WT), HCT116 DNMT1 (1KO), HCT116 DNMT3B (3BKO), and HCT116 DNMT1 + DNMT3B (DKO) knockout cells (25). Interestingly, parental HCT116 cells lacked expression of $80\%$ of the 44 (PRC1 or PRC2) + DNMT3B targets (Fig. 1B). Of the 34 genes expressed at some level in normal colon but silent in HCT116 (WT) cells, 6 were reactivated in 1KO and DKO, 7 were reactivated in 3BKO and DKO, 11 were reactivated in 1KO, 3BKO, and DKO, and 10 were reactivated only in DKO cells. Taken together, these results support our in silico analyses and suggest that use of DNMT3B and polycomb binding accurately identifies novel targets of epigenetic silencing in a colon cancer cell line. These results also show that DNMT1 and DNMT3B play both separate and overlapping roles in mediating the epigenetic silencing of these genes.

Frequent silencing and DNA hypermethylation of DNMT3B plus PcG target genes in colon cancer cell lines and primary tissues. To obtain a better estimate of the frequency of gene silencing, we examined expression of 24 of the 44 genes from Fig. 1 in a panel of eight colon tumor cell lines untreated or treated with the DNA methylation inhibitor 5-aza-dC (5 $\mu$mol/L) for 72 h. Of these 24, 20 were silenced in untreated cells and transcriptionally reactivated by 5-aza-dC-treatment in $\approx50\%$ of the lines tested (Supplementary Fig. S1; Supplementary Table S2). The majority of the 24 genes were silenced at even higher frequency (13 were silenced in 7 or 8 of 8 lines; Supplementary Fig. S1). The ability to reactivate expression by 5-aza-dC strongly suggests the involvement of DNA hypermethylation in their repression.

To directly examine the role of DNA methylation in repressing gene activity, we performed BGS on 13 of them from normal colon and HCT116 cells (Fig. 2A; Supplementary Fig. S2). For eight genes [HOXD10, JPH3, JPH4 (two regions), CHRDL1, ASCL1, FGF12, HMX2, and SIX3], DNA methylation levels were generally low in normal colon (<30%) but were densely methylated in HCT116 cells, consistent with their patterns of expression. Although expressed in normal colon, the LCK promoter was $\approx60\%$ methylated (Supplementary Fig. S2). Four other genes, SPAG6, DLGAP1, TBX4, and...
were densely hypermethylated in both normal colon and HCT116 cells, suggesting that hypermethylation is part of their normal biology (Fig. 2A; Supplementary Fig. S2). We extended the DNA methylation analysis to our full panel of seven additional colon cell lines using bisulfite pyrosequencing, revealing complete correlation between promoter DNA hypermethylation and lack of expression (Fig. 2B; Supplementary Table S2). Generally, methylation levels in excess of 40% were incompatible with transcription.

Figure 2. DNA methylation analysis of DNMT3B + PRC1/PRC2 target genes in colon tumor cell lines. A, DNA methylation patterns were determined for a subset of the genes examined in Fig. 1 using BGS. The gene promoter region is shown; bent arrow, transcription start site. Tick marks, CpG sites; thick horizontal bar (PyroSeq), region analyzed by pyrosequencing. Results are summarized below with open circles (unmethylated) or filled circles (methylated) for each CpG site. Each row is an individually cloned and sequenced molecule. Numbering is relative to the transcription start site. Left, total percent methylation over all clones and CpG sites. B, quantitative bisulfite pyrosequencing DNA methylation analysis for 12 genes in eight colon tumor cell lines. Results are presented as the percent methylation over all CpG sites within the pyrosequenced region (A; Supplementary Fig. S2). Below each graph, the expression status (Exp) of the gene, derived from Fig. 1 and Supplementary Fig. S1, is indicated; +, expressed; −, no expression.
We extended our DNA methylation and expression analyses of six genes (HAND1, HMX2, JPH3, JPH4, ASCL1, and TBX4) of the total 44 to 12 fresh-frozen primary colorectal tumor and adjacent normal tissue pairs. With the exception of TBX4, there was an excellent correlation between reduced/absent expression and DNA hypermethylation in the tumor relative to the adjacent normal tissue (Fig. 3; Supplementary Table S2; representative programs are provided in Supplementary Fig. S3). TBX4 was an interesting exception because it was not expressed in normal colon but was reexpressed in 7 of 12 tumor tissues with a concomitant small but significant reduction in methylation. We also used an extensive microarray expression database to further analyze expression of select genes (HAND1, JPH3, DLGAP1, CHRD1, ROBO1, ILIR2, HOXD10, and LCK) in silico (26). Decreased expression of all eight was observed in colorectal tumors (or metastasis) relative to normal tissue (or the primary site) in at least one microarray study (Supplementary Fig. S4). Furthermore, significant down-regulation occurred in other tumor types, suggesting that epigenetic silencing of these genes is not restricted to colon cancer. Taken together, reduced expression of genes in tumor relative to normal tissue is suggestive of a growth-suppressive role (tumor suppressors), whereas the findings for TBX4 indicate possible oncogenic function.

**DNMT3B + PRC1/PRC2 target genes regulate cell growth.** Most of the genes we identified as targets of epigenetic silencing have not been shown previously to influence colon tumor cell growth. To investigate this more directly, we cloned the full-length open reading frames of ASCL1, CHRD1, HAND1, HMX2, JPH3, SIX3, and TBX4 into an expression vector and transfected them into HCT15 cells (where the endogenous copy of all of these genes is epigenetically silenced; Supplementary Fig. S1). Ectopic expression of HAND1, HMX2, and SIX3 caused significant growth suppression (Fig. 4). In contrast, expression of TBX4 resulted in a small but significant...
increase in cell growth, consistent with its possible role as a proto-oncogene. Similar results were obtained with HCT116 cells (data not shown). Taken together, these experiments show that a subset of the genes we identified possesses properties consistent with them acting as tumor suppressors, whereas the others may regulate aspects of differentiation or cell motility that are not detectable in a colony formation assay.

Role of specific DNMTs in modulating aberrant methylation and the histone code at PRC1/PRC2 + DNMT3B target genes. Based on our expression studies (Fig. 1B), we identified genes regulated by the activity of DNMT1, DNMT3B, or both. We are particularly interested in the role of DNMT3B in gene regulation because of our prior work with ICF syndrome; therefore, we examined the HAND1, IL1R2, and ROBO1 gene promoters because they were clear DNMT3B-regulated genes in HCT116 cells (Fig. 1B) and are established targets of PRC1 and PRC2 (27–32). BGS revealed that the HAND1 and IL1R2 CpG islands (Fig. 5A and B) were unmethylated in normal colon but hypermethylated in both HCT116 and 1KO cells. DNA methylation was reduced overall in 3BKO cells and nearly absent in DKO cells at both promoters (Fig. 5). Interestingly, there was a clearly defined region upstream of the HAND1 transcription start site that lost DNA methylation in 3BKO cells. HAND1 transcription start site that lost DNA methylation in 3BKO and nearly absent in DKO cells at both promoters (Fig. 5). In 3BKO cells, activating H3 acetylation and trimethylated H3K4 marks were elevated at HAND1, whereas repressive H3K9 trimethylation and H3K27 dimethylation were reduced (Fig. 5C). Interestingly, however, H2AK119 mono-ubiquitination at HAND1 was completely lost in 3BKO cells, PRC2 binding, although reduced, was still clearly present. Our findings with HAND1 are consistent with it being a well-established target of PRC1 repression (34). Similar results for PRC2 and H2AK119 ubiquitination were observed at the IL1R2 and ROBO1-A promoters (Fig. 5D; Supplementary Fig. S5B).

Given that loss of DNMT3B function in B cells resulted in not only gene-specific but also global changes in the histone code (21), we examined total levels of select histone marks and PRC1/PRC2 subunits by Western blotting in HCT116 cells. Notable changes include reduced H3K4 dimethylation and elevated H3K9 trimethylation and acetylation in 1KO and 3BKO cells (Fig. 6A). Levels of PRC2 subunits increased in DNMT KO cells. Interestingly, some PRC1 subunits showed elevated expression (RING1/2 H2AK119 ubiquitin E3 ligases in all KOs and MEL-18 in 3BKO and DKO), whereas BMI1 expression was markedly reduced in KO cells (Fig. 6A). Increased expression of EZH2, SUZ12, RING1, RING2, and MEL-18 at the protein level could be accounted for, at least in part, by elevated mRNA levels (Supplementary Fig. S6). Interestingly, however, BMI1 mRNA levels did not parallel their respective protein levels (Fig. 6A; Supplementary Fig. S6). Taken together, these results indicate that DNMT1 and DNMT3B directly or indirectly regulate certain facets of the histone code globally (particularly H3K9 methylation and acetylation) and levels of both PRC1 and PRC2 subunits at the mRNA and/or protein levels.

We were intrigued by the marked loss of H2AK119 mono-ubiquitination in 3BKO cells at the three promoters examined thus far; therefore, we examined this mark in greater detail at 14 other genes from Fig. 1B. Ubiquitinated H2A is a repressive mark that blocks
transcriptional elongation, but not assembly, of RNA pol II at promoters (35). For 11 of 14 promoters, H2AK119 monoubiquitination was markedly or completely lost in 3BKO cells (Fig. 6B) despite global levels of this mark being relatively unchanged (Fig. 6A). This association was observed even if the gene did not become transcriptionally reactivated in 3BKO cells (e.g., DLGAP1 and JPH4; Figs. 1B and 6B; Supplementary Fig. S7), strongly suggesting that DNMT3B inactivation causes Ub-H2A loss rather than Ub-H2A loss being an indirect consequence of gene reactivation. For the remaining three promoters, no Ub-H2A was observed in any of the examined samples, indicating that they are targets of PRC2 but not of PRC1 (Fig. 6B-D; Supplementary Fig. S7). We also compared levels of PRC2 (SUZ12) binding and its mark, H3K27 trimethylation (Fig. 6C and D), and two marks of transcriptional activity/permissiveness, H3K9/K18 acetylation and H3K4 trimethylation (Fig. 6E and F). H3K27 trimethylation was reduced at 4 of 14 genes in 1KO cells and 2 of 14 genes in 3BKO cells but was lost at almost all genes in DKO cells. Similar results were observed for SUZ12, although there were clearly

Figure 5. Analysis of DNA methylation and the histone code at genes regulated by DNMT3B + PRC1/PRC2 reveals novel connections between H2A ubiquitination and DNMT3B. A, BGS analysis of the HAND1 promoter defines a region of DNA methylation mediated by DNMT3B (boxed). Labeling is as in Fig. 2A. Percent methylation within the boxed region only is underlined. B, BGS analysis of the IL1R2 promoter. C, chromatin immunoprecipitation (ChIP) followed by semiquantitative RT-PCR for the histone marks, PcG proteins, and DNMTs listed at the left of the gel photos for HAND1. D, chromatin immunoprecipitation analysis of the IL1R2 promoter. Expression status of each gene is derived from Fig. 1B. Me, methyl; 2x, dimethylated; 3x, trimethylated; Ac, acetylated; Ub, monoubiquitin; NC, normal colon.
cases where SUZ12 remained bound despite reduced H3K27 methylation. Levels of H3 acetylation and H3K4 trimethylation generally paralleled transcriptional activity and each other (Fig. 6F and F). Analysis of a more complete set of histone marks for genes in Fig. 6 is presented in Supplementary Fig. S7. Another interesting observation for several genes (e.g., HAND1; Figs. 5 and 6; Supplementary Fig. S7) was that loss of one DNMT reduced binding of the other, suggesting that interactions among DNMTs (36) regulate their targeting. We corroborated our results from HCT116 KO cells by treating parental HCT116 cells with 5-aza-dC, which inactivates their targeting. We corroborated our results from HCT116 KO cells by treating parental HCT116 cells with 5-aza-dC, which inactivates their targeting.

Discussion

In the present article, we made use of our previously identified DNMT3B-regulated genes derived from an ICF syndrome model system, combined with published PRC1 and PRC2 targets derived from ChIP-chip and ChIP-seq studies, to examine the relationships between DNMT and PcG complex binding, genes methylated in colon cancer, and the histone code. Nearly 50% of DNMT3B target genes in B cells are bound by PRC1 or PRC2 in embryonic stem cells or embryonic fibroblasts. Using DNMT3B regulation and PRC1 and/or PRC2 binding as criteria for predicting genes, we identified based on in silico analyses are indeed bound by DNMT3B and PRC2 and are PRC1-marked in normal colon. In addition, our results suggest that DNMT3B is specifically involved in recruiting and/or maintaining ubiquitinated H2A at certain gene promoters.

3BKO cells flanking the transcription start sites and concomitant changes in the histone code. Interestingly, whereas loss of DNMT1 or DNMT3B resulted in marked changes in certain histone marks globally and in the levels of PRC1 and PRC2 complex subunits, especially BMI1 and MEL-18, the most consistent alteration at specific gene promoters in 3BKO cells was loss of H2AK119 monoubiquitination, a PRC1 complex-mediated mark. Taken together, these results show that the complement of epigenetic marks and DNMT3B binding in normal cells may serve as a good predictor of genes susceptible to acquiring aberrant DNA hypermethylation in cancer and that DNMT3B in particular plays a role in the activity and/or recruitment of PRC1 in differentiated cells.

Our findings show that use of DNMT3B regulation, in addition to PRC1 and/or PRC2 binding as criteria for predicting genes that become hypermethylated in colon cancer, yields a high rate of positive hits. Further analysis of DNMT binding throughout the genome is therefore clearly warranted. Most of the genes we characterized have not been well studied in the context of colon cancer. Those acquiring aberrant methylation, such as the basic helix-loop-helix transcription factors ASCL1 and HAND1, may play important roles in regulating transcription of other genes that suppress growth or maintain the differentiated state. HAND1, which is expressed in developing and mature gut, may regulate expression of sonic hedgehog and the homeobox gene IRX4 (37). TBX4 is an interesting case where DNA methylation is involved in suppressing its expression in normal colon. Whereas little is known about TBX4 in the context of cancer, TBX3 is highly overexpressed in breast cancer and represses the ARAF tumor suppressor (38), supporting our hypothesis that TBX4 acts as an oncogene. CHRD1L encodes a secreted BMP antagonist selectively expressed in colon crypts. Its epigenetic silencing may therefore negatively affect intestinal stem cell differentiation (39). Another group has also reported HAND1 methylation in colon cancer, consistent with our findings (40). Homeobox genes HOXD10,
SIX3, and PROX1 were recently shown to be hypermethylated in lung cancer (41). Clearly, further study of how these genes regulate growth and differentiation of normal and transformed colon cells is warranted.

Here, we have focused on how the activities of DNMT1 and DNMT3B are involved in regulating the histone code and PcG protein binding in tumor cells once methylation has been established. Although histone methylation may initially recruit DNA methyltransferase (11), once the densely hypermethylated state characteristic of tumor suppressor genes in cancer cells is established, our data suggest that DNMT3B is required for maintenance of H2AK119 monoubiquitination at a subset of polycomb targets. This may be mediated by direct physical contacts between the two complexes or indirectly via the DNA methylation mark itself. As we were unable to reliably perform chromatin immunoprecipitation for PRC1 with commercially available antibodies, we cannot yet determine if lack of DNMT3B interferes with PRC1 recruitment or with PRC1 enzymatic activity once recruited. DNMTs may bind to promoter regions in normal cells even if they are unmethylated, consistent with our previous ICF syndrome study (21). PcG complexes also bind to transcriptionally active or competent genes (28). DNMT3B may be kept catalytically inactive by PRC1 perhaps to have it poised for later de novo methylation-mediated repression or DNMT3B participates in repression independent of methylation in this state. A shift to de novo methylation could occur due to changes in PRC1 complex subunit composition or post-translational modifications of PcG complex subunits or of DNMT3B itself. We observed reduced BMI1 subunit composition or post-translational modifications of PcG complexes. Although histone methylation may initially recruit DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999;99:247–57.

It remains a fundamental unanswered question why certain genes become hypermethylated in cancer. Recent findings showing that PcG target genes in normal cells are more likely to acquire aberrant promoter hypermethylation in cancers (17, 18, 24), along with evidence of interactions between DNMT1/DNMT3B and several PcG complex subunits (EZH2 and BM1; refs. 12, 42), are beginning to shed light on how this process may occur. Genes hypermethylated in cancer cells adopt a bivalent pattern of histone marks and low-level expression (characteristic of developmental genes in embryonic stem cells) when treated with DNA methylation inhibitors (43), lending further support for a mechanistic connection. Our results indicated that 5-aza-dC treatment altered histone marks in much the same way as DNMT1 and DNMT3B double knockout. Other data showing that the EZH2-DNMT targeting process may not be universal (44) and that the association between tumor-specific DNA hypermethylation events and PcG binding may be cell type specific (20, 45) show that we still have much to learn. It was striking that most PcG targets we examined lost H2AK119 monoubiquitination on disruption of DNMT3B but not DNMT1. This despite the observation that DNMT1 loss results in marked reduction in BM1 levels. Therefore, DNMT1 and DNMT3B may play distinct roles in modulating PRC1 function. Interestingly, DNMT1 is essential for maintenance of and recruitment of BM1 to PcG bodies (46). DNMT3B was not examined in this study, although PcG bodies are known to colocalize with pericentromeric satellite DNA (47), an established DNMT3B target region. Understanding the full spectrum of connections between DNMT3B, H2A ubiquitination, and PRC1 will likely require the use of genome-wide approaches as part of future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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