Down-regulation of Human DAB2IP Gene Expression Mediated by Polycomb Ezh2 Complex and Histone Deacetylase in Prostate Cancer*

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Human DAB2IP (hDAB2IP), a novel GTPase-activating protein modulating the Ras-mediated signaling and tumor necrosis factor-mediated apoptosis, is a potent growth inhibitor in human prostate cancer (PCa). Loss of hDAB2IP expression in PCa is due to altered epigenetic regulation (i.e. DNA methylation and histone modification) of its promoter region. The elevated polycomb Ezh2, a histone methyltransferase, has been associated with PCa progression. In this study, we have demonstrated that an increased Ezh2 expression in normal prostatic epithelial cells can suppress hDAB2IP gene expression. In contrast, knocking down the endogenous Ezh2 levels in PCa by a specific small interfering RNA can increase hDAB2IP expression. The association of Ezh2 complex (including Eed and Suz12) with hDAB2IP gene promoter is also detected in PCa cells but not in normal prostatic epithelial cells. Increased Ezh2 expression in normal prostatic epithelial cells by cDNA transfection facilitates the recruitment of other components of Ezh2 complex to the hDAB2IP promoter region accompanied with the increased levels of methyl histone H3 (H3) and histone deacetylase (HDAC1). Consistently, data from PCa cells transfected with Ezh2 small interfering RNA demonstrated that reduced Ezh2 levels resulted in the dissociation of Ezh2 complex accompanied with decreased levels of both methyl H3 and HDAC1 from hDAB2IP gene promoter. We further unveiled that the methylation status of Lys-27 but not Lys-9 of H3 in hDAB2IP promoter region is consistent with the hDAB2IP levels in both normal prostatic epithelial cells and PCa cells. Together, we conclude that hDAB2IP gene is a target gene of Ezh2 in prostatic epithelium, which provides an underlying mechanism of the down-regulation of hDAB2IP gene in PCa.

The human DOC-2/DAB2 interactive protein gene (hDAB2IP) located at chromosome 9q33.1-33.3 is a new member of the Ras GTPase-activating family gene (1, 2). Our recent data indicate that hDAB2IP protein is a growth inhibitor in prostate cancer (PCa)3 cells (3). In addition, hDAB2IP protein (also named ASK-interacting protein 1 (AIP1)) is involved in the tumor necrosis factor-mediated JNK signaling pathway leading to cell apoptosis (4, 5). We have demonstrated that normal prostatic epithelial cells express higher hDAB2IP levels than PCa cells, which is due to epigenetic alteration (i.e. aberrant DNA methylation and histone deacetylation) in the promoter region during carcinogenesis. Similarly, loss of hDAB2IP expression was also detected in breast and lung cancer specimens (6, 7) frequently associated with the promoter hypermethylation.

Human enhancer of Zeste homolog (Ezh2) protein belongs to Polycomb repressive complex 2/3 (8), which also includes Eed, Suz12, and the histone-binding protein RbAp48/46 (9–13). The Ezh2 complex appears to be a transcription repressor that has been shown to be involved in cellular memory system, X-inactivation, germline development, stem cell pluripotency, and cancer metastasis (14–20). This complex exhibits an intrinsic histone lysine methyltransferase activity on histone H3 Lys-27 and 9 or histone H1 Lys-26 mediated by the SET domain of Ezh2 (8, 10, 11, 21, 22).

Recent data indicate that elevated Ezh2 levels are found in hormone-refractory, metastatic PCa (23, 24) as well as in poorly differentiated breast carcinomas (25, 26). However, the underlying mechanism of Ezh2 in these cancer cells is still unknown. In this study, we found an inverse correlation between Ezh2 and hDAB2IP gene expression in either normal prostatic epithelia or PCa cells. Increased Ezh2 expression in normal prostatic epithelial cells could inhibit the hDAB2IP promoter activity and its gene expression. In contrast, knockdown of Ezh2 expression by siRNA in PCa cells resulted in an elevated hDAB2IP gene expression. These data prompted us to investigate the role of Ezh2 in modulating hDAB2IP gene expression, and we demonstrated that Ezh2 complex and histone deacetylase (HDAC) are associated with hDAB2IP promoter regions in PCa cells but not in normal prostatic epithelial cells. The outcome of this study provides an underlying mechanism of the functional role of Ezh2 in metastatic PCa.

EXPERIMENTAL PROCEDURES

Cell Cultures—Three human prostate cancer cell lines (LNCaP, C4-2, and PC3) were maintained in T medium supplemented with 5% fetal bovine serum (27). MDAPCa 2a and MDAPCa 2b cell lines derived from patients with bony metastasis (28) were maintained in BRFF-HPC1 medium (Biological Research Faculty and Facility, Inc., Jamsville, MD) supplemented with 20% fetal bovine serum. A VCAP cell line derived from a vertebral metastatic lesion of prostate cancer (29) and DU145 were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. Three normal human prostate epithelial cells (PrEC1, PrEC2, and PrEC3) were maintained in chemical-defined medium (PrEGM) purchased from Cambrex. PZ-HPV-7 (an immortalized transition; qRT-PCR, quantitative RT-PCR; ChIP, chromatin immunoprecipitation; SET, Su-(var) 3–9; E(z), Trithorax.

This paper is available on line at http://www.jbc.org

Received for publication, February 7, 2005, and in revised form, March 29, 2005
Published, JBC Papers in Press, April 6, 2005, DOI 10.1074/jbc.M501379200

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Printed in U.S.A.
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cell line derived from the peripheral zone of a normal prostate (30, 31) and three additional primary prostatic epithelial cells (SWPC1, SWPC2, and SWPC3) (derived from cancer lesions), and SWNPC2 (derived from the adjacent normal tissue) were maintained in PrEGM medium.

**Transient Transfection and Luciferase Reporter Gene Assay**—Cells were plated at a density of 1.8 × 10^5 cells/well in a 6-well plate. After 24 h, PrEC1 and PZ-HPV-7 cells were co-transfected with Myc-tagged human Ezh2 expression construct (32) and a luciferase reporter vector containing the hDAB2IP promoter-pGL3-P2 (2) using Lipofectamine Plus transfection reagent (Invitrogen). Luciferase assay was described previously (1, 2). All experiments were repeated at least three times in triplicate. The relative luciferase activity was calculated as described previously (2).

**RNA Isolation and Quantitative RT-PCR (qRT-PCR)**—Total cellular RNA was isolated using an RNaasy kit (Qiagen, Inc.) according to the manufacturer’s instructions. To measure hDAB2IP or Ezh2 mRNA levels, 1 μg of total cellular RNA from each cell line was reversibly transcribed into first strand cDNA using iScript™ cDNA synthesis kit (Bio-Rad).

The first strand cDNA was further amplified by qRT-PCR using hDAB2IP primer set F-hDAB2IP, 5'-TGGACGATGTGGTACCTATGCCC-3'; R-hDAB2IP, 5'-GGATGGTGATGGTTTGGTAG-3' and Ezh2 primer set F-Ezh2, 5'-GGCGAAGCTGAGTGAATACCTGC-3'; or R-Ezh2, 5'-GGCGAAGCTGAGTGAATACCTGC-3'. The reactions were carried out in a 96-well plate, and PCR amplification protocol was performed as follows: 95 °C (1 min), followed by 40 cycles of 95 °C (15 s), 55 °C (1 min), 72 °C (1 min) using an iCycler iQ machine (Bio-Rad). The 18S cDNA (F-18S, 5'-CCGGAATTGACGGAAGGGCACCAC-3', R-18S, 5'-GGTGCAGCCCCGGACATCTAAGG-3') was used as an internal control. All experiments have been repeated twice in duplicates. hDAB2IP or Ezh2 mRNA level was determined by normalizing with the 18 S cDNA of each sample.

The quality control was carried out using both electrophoresis analyses on a 2% NuSieve agarose gel (3:1, FMC Bioproducts) and melting curve analysis performed immediately after the end of amplification. The quality control was carried out using both electrophoresis analyses on a 2% NuSieve agarose gel (3:1, FMC Bioproducts) and melting curve analysis performed immediately after the end of amplification.

**Western Blot Analysis**—Cells were lysed with whole cell lysis buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM MgCl_2, 0.5 mM EDTA, 10% glycerol, 1% Triton X-100, 10 mM NaF, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) and were alternately frozen and thawed to rupture the cell membranes. Samples were incubated for 30 min on ice to lyse the nuclei and then centrifuged at 4,000 × g for 5 min to pellet the cell membranes. The protein concentration of each sample was determined by a standard Bradford assay. Equal amounts of protein (20 μg) of each cell line were subjected to Western blot analysis. Antibodies used for probing were hDAB2IP (3), DOC-2/DAB2 (BD Biosciences), Lamin A/C (Upstate Biotechnology), Ezh2 (Upstate Biotechnology), and actin (Sigma).

**RNA Interference**—Two different 21-nucleotide duplex siRNAs for Ezh2, (5'-AAGAGGTTGACGAGCGGTATG-3') (23), 5'-AAGACCTCT-GAATGCTGGTTCG-3') (33), and control siRNA for Lamin A/C (5'-CATG-GACCTCAGAGACCACCACTG-3') were synthesized by Qiagen. Twenty-four hours after plating, the cells were transfected either with both Ezh2, siRNA duplexes together (100 nmol each) or with control siRNA (200 nmol) using RNAiFeet transfection reagent (Qiagen) according to the manufacturer’s instructions. At various time points after transfection, the cells were harvested and subjected to Western blot analysis.

**Chromatin Immunoprecipitation (ChIP) Assay**—This assay for hDAB2IP gene promoter was performed as described previously (2). For hDAB2IP gene promoter, the same PCR condition was applied with two primer sets as follows: first PCR (5'-ACAATCCTGAGCAGTGGC-3' and 5'-GGCGGAGGATACTTGTC-3') and second PCR (5'-CTCAG-CGGAGCTCAGGGAG-3' and 5'-TCTGAGTACCTCCCAAGTTTGGTCCAGTTG-3'). Brieﬂy, precleared chromatin from 2 × 10^5 cells was used for each ChIP assay, and 5 μg of each antibody was used in this assay including Ezh2, Suz12, trimethyl H3 (Lys-27), trimethyl H3 (Lys-9), and dimethyl H3 (Lys-9) purchased from Abcam Inc.; and Eed, dimethyl H3 (Lys-27), monomethyl H3 (Lys-27), acetyl H3, and HDAC1 purchased from Upstate Biotechnology. The total input or immunoprecipitated DNA was determined by qPCR at least twice with the equation ΔΔCt (threshold cycle) of each sample = mean of C_(input) - mean of C_(chip).

**RESULTS**

Profiling hDAB2IP and Ezh2 Expression in Various Prostatic Epithelia—To evaluate the expression profile of hDAB2IP gene and Ezh2 in the prostate cell lines, we performed qRT-PCR to document the steady-state levels of hDAB2IP (Fig. 1A) and Ezh2 (Fig. 1B) mRNA in cells derived from normal prostatic epithelium (PrEC1, PrEC2, PrEC3, PZ-HPV-7, and SWNPC2), primary PCa cells (SWPC1, SWPC2, and SWPC3), or metastatic PCa cell lines (LNCaP, C4-2, PC3, MADPC2a, MADPC2b, VCAP, and DU145). We observed a trend of decreasing hDAB2IP mRNA from normal cells to malignant cells; however, an opposite trend of Ezh2 mRNA was seen from normal cells to malignant cells. Western blot data (Fig. 1C) also confirmed the qRT-PCR results. Taken together, an inverse correlation between Ezh2 and hDAB2IP gene expression prompted us to investigate the possibility that hDAB2IP gene expression could be modulated by the Ezh2 complex particularly, since loss of hDAB2IP expression was frequently observed in metastatic PCa cells.

The Inhibitory Effect of Ezh2 on hDAB2IP Gene Expression—To test whether Ezh2 is a negative regulator to modulate hDAB2IP gene expression in PCa cells, Western blot analysis was performed using whole cell extract prepared from PCa cells transfected with either a mixture of two different Ezh2 siRNAs (23, 33) or lamin A/C siRNA. The significantly decreased Ezh2 levels in PC3 (Fig. 2A) and in DU145 (Fig. 2C) were observed within 48–72 h after transfection, and the reduced Ezh2 protein became more prominent 96 h after transfection. Under this condition, the elevated levels of hDAB2IP protein were detected in both PCa cells. In contrast, Lamin A/C siRNA duplex did not alter Ezh2 or hDAB2IP levels in either cell type (Fig. 2, B and D). To demonstrate the specific effect of Ezh2 on hDAB2IP gene expression, the DOC-2/DAB2 gene (27), which is not regulated by Ezh2 in prostate (Fig. 2), was used as a negative control in this study.

To determine the inhibitory effect of Ezh2 on hDAB2IP gene expression via the transcriptional or post-transcriptional regulation, we examined the hDAB2IP promoter activity in several normal prostatic epithelial cells by transfecting with both pGL3-P2 and Ezh2 expression vectors. We observed that an increased Ezh2 protein expression (Fig. 3A) in normal prostatic epithelial cells could inhibit the hDAB2IP promoter activity (Fig. 3, B–E) in a dose- and time-dependent manner. Using qRT-PCR, we also observed a similar inhibitory effect of Ezh2 on hDAB2IP mRNA levels in these and two other normal epithelial cells such as PrEC2 and PrEC3 (data not shown). We also generated a stable Ezh2-expressing PZ-HPV-7 transfectant and found reduction of the endogenous hDAB2IP protein levels in these cells (Fig. 3F). These data clearly indicate that the suppression of hDAB2IP gene promoter activity is mediated by Ezh2 protein.

The Association between Ezh2 Complex and HDAC1 in hDAB2IP Promoter Region in PCa Cells—To demonstrate the direct interaction of Ezh2 complex with hDAB2IP promoter region, ChIP assay was performed. In Fig. 4A, robust binding of Ezh2 as well as two other components, Eed and Suz12, to the hDAB2IP promoter was seen in PC3 cells, the moderate binding of Ezh2 complex was detected in DU145 cells, and the lowest binding of Ezh2 complex was detected in PZ-HPV-7 cells. Noticeably, the amount of Ezh2 complex associated with hDAB2IP promoter inversely correlated with hDAB2IP mRNA and protein expression patterns in these cells (Fig. 1). On the other hand, we did not observe an inverse correlation between Ezh2 and DOC-2/DAB2 levels in these cells (Fig. 1C) or a consistent association pattern between Ezh2 complex and DOC-2/DAB2 gene promoter (Fig. 4).
It is known that transcriptional repression mediated by human polycomb group (PcG) protein involves histone deacetylation (34). We therefore determined the association of HDAC1 with the \textit{hDAB2IP} promoter region. As shown in Fig. 4B, the higher levels of HDAC1 associated with \textit{hDAB2IP} promoter were detected in PC3 and DU145 cells than in PZ-HPV-7 cells. In contrast, the lower levels of acetyl H3 associated with \textit{hDAB2IP} promoter were seen in these two PCa cells than in PZ-HPV-7 cells (Fig. 4B). These data indicate that this repression complex associated with \textit{hDAB2IP} promoter contains both Ezh2 complex and HDAC1.

Furthermore, by increasing Ezh2 gene expression in PZ-HPV-7 cells, we observed that the presence of Ezh2 protein could recruit not only other components of PcG proteins such as Eed and Suz12 (Fig. 5A) but also HDAC1 to the \textit{hDAB2IP} promoter region in these cells. Meanwhile, the decreased acetyl H3 level became more apparent in these cells (Fig. 5B). Taken together, Ezh2 plays a key role in recruiting other transcription repressors to the \textit{hDAB2IP} promoter region in prostatic epithelia. On the other hand, we were able to show the dissociation of both PcG complex and HDAC1 from \textit{hDAB2IP} gene promoter in PC3 (Fig. 6) and DU145 (Fig. 6) when their endogenous Ezh2 levels were knocked down by Ezh2 siRNA (Fig. 2). Under the same condition, the acetyl H3 levels were elevated. For DOC-2/DAB2 gene, no consistent pattern of these complexes could be observed in the promoter region (Figs. 4 and 5). Thus, these data further support the notion that \textit{hDAB2IP} is an Ezh2 target gene.

The Methylation Status of H3-Lys-9 or H3-Lys-27 in \textit{hDAB2IP} Promoter Region Modulated by Ezh2—Although the human Eed-Ezh2 complex and its \textit{Drosophila} ESC-E(Z) counterpart have been shown to be HMKTase (9), its substrate specificity still remains unclear. For example, Cao et al. (11) and Muller et al. (22) showed that Lys-27 in H3 is the only amino acid methylated by Ezh2; however, other groups (10, 21) demonstrated that Ezh2 can also methylate H3-Lys-9. Moreover, the lysine in H3 exists in mono-, di-, and tri-methyl status (35–37), and their impact on gene regulation is still unclear. For \textit{hDAB2IP} promoter region, di- and tri-methyl H3-Lys-27 were highly detectable in PC3 and DU145 cells but not in PZ-HPV-7 (Fig. 4C), which is consistent with the presence of Ezh2 complex in these three cell lines (Fig. 4A). We also noticed that the levels of mono-methyl H3-Lys-27 did not vary among these three cell lines and did not change in the presence of elevated Ezh2 (Figs. 4C and 5C), suggesting that mono-methyl H3-Lys-27 may reflect a “constant basal” methylation status of
Lys-27. Caretti et al. (38) have reported a similar observation in the study of MHCIIb and MCK gene regulation during muscle differentiation. By increasing Ezh2 protein expression in PZ-HPV-7 cells, we demonstrated that the levels of both di- and tri-methyl but not mono-methyl H3-Lys-27 elevated dramatically in a dose-dependent manner (Fig. 5C). In the presence of Ezh2 siRNA in either PC3 or DU145 cells, the decreased levels of both di- and tri-methyl H3-Lys-27 could be observed (Fig. 6). These data suggest that the effect of Ezh2 is to convert H3-Lys-27 into tri-methyl status in the hDAB2IP gene promoter region.

On the other hand, for the methylation status of H3-Lys-9, we failed to detect any good correlation among these three cell lines tested. For example, tri-methyl H3-Lys-9 was highly detectable in these cell lines, and di-methyl Lys-9 status was not consistent with hDAB2IP levels among these three cell lines (Fig. 4D). Moreover, increased Ezh2 expression in PZ-HPV-7 cells was associated with a dramatic elevation of di-methyl but not tri-methyl H3-Lys-9 (Fig. 5D), suggesting that the methylation status of H3-Lys-9 may not be involved in modulating hDAB2IP gene promoter activity by Ezh2. Together, we believe that hDAB2IP gene silencing in Pca can be mediated by the elevated levels of Ezh2, which results in the hypermethylation of H3-Lys-27 and the recruitment of HDAC. This study provides an underlying mechanism of the functional role of Ezh2 in metastatic Pca.

![FIG. 2. The effect of Ezh2 on hDAB2IP protein expression in Pca cells.](http://www.jbc.org/)

PCa is a leading cause of cancer-related death in males and is second only to lung cancer. Although effective surgical and radiation treatments are available for clinically localized Pca, metastatic Pca remains essentially incurable. To understand the biology of metastatic Pca, data from a cDNA microarray analysis indicate that elevated Ezh2 with histone lysine methyltransferase activity is often associated with metastatic Pca but not with benign tissue and organ-confined tumor (23). Noticeably, clinically localized prostate cancer expressing higher levels of Ezh2 often has a poorer prognosis (24), suggesting that Ezh2 is not only a potential biomarker for predicting the relative risk of Pca progression but also a key contributor for the disease progression. Here we have demonstrated that Ezh2 mRNA and protein are more consistently elevated in metastatic Pca cells than in normal cells. The pattern of hDAB2IP levels was detected in PCa cells transfected with Ezh2 siRNAs (Fig. 2). In contrast, the reduced hDAB2IP gene expression was observed in normal prostatic epithelial cells transfected with Ezh2 expression vector (Fig. 3). These results provide an important mechanism of the elevated Ezh2 expression during Pca progression.
In Drosophila, ESC-E(Z) proteins recognize and exert their activity through specific DNA sequences known as polycomb response elements (39–41). In mammals, such elements have not been identified yet. It remains unclear how mammalian PcG complexes are recruited to chromatin to regulate expression of a specific target gene. Because most of the PcG proteins do not contain a DNA-binding domain, target genes cannot be identified by searching the consensus-binding site in the genome (42). Thus, the ChIP assay can provide direct evidence for the possible interaction between the Ezh2 complex and promoter region of any known gene (43). Our data indicate that the levels of Ezh2 associated with the hDAB2IP promoter region inversely correlate with the steady-state levels of hDAB2IP mRNA and protein in all three cell lines tested. Most importantly, Ezh2 plays an active role in recruiting other PcG components such as Eed and Suz12 to form a functional complex along with HDAC1 to the promoter region of hDAB2IP gene (Figs. 5 and 6). Based on these results, one could predict that the presence of elevated Ezh2 levels in PCa signifies the downregulation of tumor suppressor genes via the cooperative effect of histone methylation and deacetylation. This study provides further evidence to support the critical role of epigenetic gene regulation in PCa progression.

In mammals, such elements have not been identified yet. It remains unclear how mammalian PcG complexes are recruited to chromatin to regulate expression of a specific target gene. Because most of the PcG proteins do not contain a DNA-binding domain, target genes cannot be identified by searching the consensus-binding site in the genome (42). Thus, the ChIP assay can provide direct evidence for the possible interaction between the Ezh2 complex and promoter region of any known gene (43). Our data indicate that the levels of Ezh2 associated with the hDAB2IP promoter region inversely correlate with the steady-state levels of hDAB2IP mRNA and protein in all three cell lines tested. Most importantly, Ezh2 plays an active role in recruiting other PcG components such as Eed and Suz12 to form a functional complex along with HDAC1 to the promoter region of hDAB2IP gene (Figs. 5 and 6). Based on these results, one could predict that the presence of elevated Ezh2 levels in PCa signifies the downregulation of tumor suppressor genes via the cooperative effect of histone methylation and deacetylation. This study provides further evidence to support the critical role of epigenetic gene regulation in PCa progression.

The term “epigenetic” refers to the heritable changes in gene expression that are caused by mechanisms other than the alteration in the nucleotide sequence. This concept generates tremendous new knowledge in understanding gene expression in mammalian cells. In addition to DNA methylation, increas-
ing studies have focused on the impact of covalent modifications of the histone core in the nucleosome structure on gene regulation. One of the covalent modifications, lysine methylation, has emerged as an important player in regulating gene expression and chromatin function. Lysine methylation usually occurs on the Lys-4, Lys-9, Lys-27, Lys-36, and Lys-79 of H3.

**FIG. 4.** The status of PcG complex, HDAC1, histone methylation, and histone acetylation on the hDAB2IP promoter region in prostatic epithelia. A ChIP assay was performed using DNA-protein complex isolated from PZ-HPV-7 (lane 1), PC3 (lane 2), and DU145 (lane 3) cells immunoprecipitated with various antibodies. The amount of endogenous hDAB2IP or DOC-2/DAB2 gene promoter was determined by qPCR using the specific primer set described previously (2) and visualized with gel electrophoresis. The number under each lane representing the fold of enrichment was calculated as $1/2^{ΔCt_{(sample)}} - ΔCt_{(PZ-HPV-7)}$.

|          | hDAB2IP | DOC-2/DAB2 |
|----------|---------|------------|
| A        |         |            |
|          | 1       | 2          | 3          |
| Ezh2     | 1       | 51.9       | 2.3        |
| Eed      | 1       | 23.4       | 7.5        |
| Suz12    | 1       | 6.7        | 16.0       |
| Input    |         |            |            |

|          | hDAB2IP | DOC-2/DAB2 |
|----------|---------|------------|
| B        |         |            |
|          | 1       | 2          | 3          |
| Acetyl H3| 1       | 0.003      | 0.23       |
| HDAC1    | 1       | 6.5        | 12.5       |
| Input    |         |            |            |

|          | hDAB2IP | DOC-2/DAB2 |
|----------|---------|------------|
| C        |         |            |
|          | 1       | 2          | 3          |
| Trimethyl H3 (K27) | 1 | 0.38 | 0.04 |
| Dimethyl H3 (K9)    | 1 | 101.0 | 494.5 |
| Input    |         |            |            |

|          | hDAB2IP | DOC-2/DAB2 |
|----------|---------|------------|
| D        |         |            |
|          | 1       | 2          | 3          |
| Trimethyl H3 (K9) | 1 | 0.38 | 0.04 |
| Dimethyl H3 (K9)    | 1 | 101.0 | 494.5 |
| Input    |         |            |            |
the Lys-20 of H4, and the Lys-26 of H1 (35, 36, 44). Biochemical and genetic studies indicate that methylation of different lysine residues, with the exception of H3-Lys-79, is catalyzed by different proteins containing SET domain (45). Although Ezh2 protein contains a SET domain, and several groups (10, 11, 21) have shown Ezh2 with histone lysine methyltransferase activity, its substrate specificity is still controversial. In addition, methylated lysine in H3 can exist in mono-, di-, and tri-methyl

![Figure 5](http://www.jbc.org/)

**FIG. 5.** The impact of Ezh2 on the status of H3 methylation and acetylation of the hDAB2IP promoter region in PZ-HPV-7 cells after recruiting PcG complex proteins and HDAC1. A ChIP assay was performed using DNA-protein complex isolated from PZ-HPV-7 cells transfected with different amounts of Ezh2 expression vector and immunoprecipitated with various antibodies. The amount of endogenous hDAB2IP gene promoter was determined by qPCR using the specific primer set described previously (2) and visualized with gel electrophoresis. The number under each lane representing the fold of enrichment was calculated as $1/2^{\Delta\delta}(\text{Ezh2}) - \Delta\delta(\text{control})$. 

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![Diagram A](http://www.jbc.org/)

![Diagram B](http://www.jbc.org/)

![Diagram C](http://www.jbc.org/)

![Diagram D](http://www.jbc.org/)
status (35, 36), and the functional significance of these modifications is largely unknown. It appears that both di- and trimethyl H3-Lys-27 are consistent with the presence of Ezh2 in the promoter region of hDAB2IP gene from both normal prostate epithelia and PCa cells. The presence of Ezh2 can dramatically increase the levels of di- and trimethyl H3-Lys-27, implying that hypermethylation of H3-Lys-27 could be a key factor in silencing hDAB2IP gene expression. In summary, hDAB2IP gene silencing mediated by Ezh2 complex and HDAC1 provides a better understanding of the functional role of Ezh2 in metastatic PCa and perhaps in other cancer types such as breast cancer.

Acknowledgments—We thank Dr. Jenuwein for providing Ezh2 expression vector, Dr. Pienta for providing VCAP cell line, and Jennifer Stanfield for editing this manuscript.

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