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H3K27me3 is an Epigenetic Mark of Relevance in Endometriosis

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Abstract

Epigenetic mechanisms may play an important role in the etiology of endometriosis. The modification of histones by methylation of lysine residues has been shown to regulate gene expression by changing chromatin structure. We have previously shown that endometriotic lesions had aberrant levels of histone acetylation (lower) and methylation (higher) than control tissues. We aimed to determine the levels of trimethylated histone 3 at lysine residue 27 (H3K27me3), a well-known repressive mark, by immunoassay of fresh tissues and immunohistochemistry (IHC) of an endometriosis-focused tissue microarray. Also, we aimed to determine levels of expression of enhancer of zeste homolog 2 (EZH2), the enzyme responsible for trimethylation of H3K27me3, in cell lines. Average levels of H3K27me3 measured by immunoassay were not significantly different in lesions compared to endometrium from patients and controls. However, there was a trend of higher levels of H3K27me3 in secretory versus proliferative endometrium. The results of IHC showed that lesions (ovarian, fallopian, and peritoneal) and secretory endometrium from controls have higher percentage of H3K27me3-positive nuclei than eutopic endometrium from patients. Endometriotic epithelial cells express high levels of EZH2, which is upregulated by progesterone. This study provides evidence in support of a role of H3K27me3 in the pathogenesis of endometriosis and for EZH2 as a potential therapeutic target for this disease, but more studies are necessary to understand the molecular mechanisms at play.

Keywords

endometriosis, epigenetics, histone methylation, H3K27me3

Introduction

Endometriosis is an estrogen-dependent, progesterone-resistant condition that affects 1 of 10 women around the world.[1] Knowledge of the pathophysiology of endometriosis is limited, but genetic, environmental, inflammatory, and, recently, epigenetic factors are known to be involved in this disease.[2–5] Epigenetic mechanisms exert their biological effects by regulating gene expression via modulation of chromatin structure without affecting the nucleotide sequence. Several epigenetic mechanisms regulate gene expression including promoter methylation at CpG islands and distinct histone modifications including acetylation, methylation, phosphorylation, and sumoylation.[6–8]

Histone methylation is one of the key epigenetic modifications that play a role in several cellular processes via modulation (activation or silencing) of transcriptional activity. This regulation is mediated by changes in the chromatin structure to allow (or block) access to the transcriptional machinery. Specific histones can be methylated at various levels, that is, mono-, di-, or trimethylation, which are correlated with either gene expression or gene repression. Trimethylation of lysine residue 27 at histone 3 (H3K27me3) is a well-known repressive histone modification that silences gene transcription and plays a role in stem cell differentiation and carcinogenesis.[9–11] Enhancer of Zeste Homolog 2 (EZH2) is the catalytic subunit of the polycomb repressive complex 2, which catalyzes the addition of 3 methyl groups to the lysine residue 27 at histone 3.[10] High levels of H3K27me3 have been observed in different types of cancer including ovarian, prostate, breast, and colon cancers,[12,13] and its levels can be used as prognosis marker in patients with renal cell carcinoma and pediatric glioblastoma.[14,15] We have previously shown that endometriotic lesions are characterized by hypoacetylation of H3K9 and H3K16 and hypermethylation of H3K4, H3K9, and H3K27.[16,17] Based on these findings, we aimed to further study the relevance of H3K27me3 specifically in endometriosis.

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The purpose of this study was to determine the levels of H3K27me3 by immunoassay of whole tissues, immunohistochemistry (IHC) of tissue microarray (TMA), and Western blot (WB) of cell line protein extracts. We hypothesized that (1) endometriotic lesions and endometrium of patients are characterized by higher levels of H3K27me3 than control tissues (eutopic endometrium) and (2) the expression of EZH2, the histone methyltransferase (HMT) responsible for catalyzing H3K27 trimethylation, is regulated by ovarian steroid hormones in endometriotic epithelial cell lines. Our data suggest that H3K27me3 may play a role in the different mechanisms at play in endometriosis. Since histone methylases and demethylases have been proposed as potential new therapies for endometriosis,[18] these results are of great translational value.

Materials and Methods

Tissue Collection

Fresh tissues (endometriotic lesions [n = 14], eutopic endometrium from patients [n = 17], and eutopic endometrium from controls [n = 15]) were obtained from premenopausal women aged 16 to 49 years with and without endometriosis during surgery and immediately frozen at −80°C after collection. Cases are defined as women with surgically diagnosed endometriosis and controls were women undergoing surgery for benign gynecologic conditions (ie, fibroids) who were found not to have endometriosis during surgery. The tissues were analyzed by histopathology to confirm endometriosis, defined as the presence of endometrial glands and stroma, and if is endometriosis, to date the menstrual cycle phase according to Noyes.[19] Demographic profiles of patients used for detection of H3K27me3 status by enzyme-linked immunosorbent assay in the study are presented in Table 1.

Cell Culture and Ovarian Steroid Hormone Treatments

Human epithelial cells EEC/MCF-7, a nonendometriotic epithelial cell line, and human endometriotic epithelial cells (12Z; gifts from Dr A. Fazleabas and Dr A. Starzinski, respectively) were used for the experiments.[20] The EEC/MCF-7 cells were cultured in Dulbecco Modified Eagle Medium (DMEM)/F-12 Nutrient Mixture supplemented with 10% fetal bovine serum (FBS), 1% ampicillin/streptomycin antibiotic, 160 ng/mL of bovine insulin at 37°C, and 5% CO_2. The 12-Z cells were grown in DMEM/F12 with phenol red complete media supplemented with 10% FBS, 1% ampicillin/streptomycin antibiotic, and 1% of sodium pyruvate. The EEC/MCF-7 and 12Z cells were grown to confluence in normal complete media and split into 25 cm^2 tissue culture flasks at a cell density of 1.0×10^6 cells. Once cells reached 85% confluence, they were cultured in deprived media for 24 hours. On day 1, cells were incubated with physiological levels (10^{-8} mol/L of estrogen [E_2] and 10^{-7} mol/L of progesterone [P_4]) of steroid hormones, alone or in combination, diluted in deprived media. Control cells received only deprived media.

Protein Extraction and WB Analysis

Briefly, 90% confluent cells growing in log phase were washed with 10 mmol/L cold PBS followed by a centrifugation of 1500 rpm for 5 minutes at 4°C. Then, cell pellet was washed again with for 10 mmol/L cold PBS followed by a centrifugation of 1500 rpm for 5 minutes at 4°C. Cell pellets were lysed by resuspending the pellet in lysis buffer (50 nmol/L Tris-HCl [pH 7.5], 150 mmol/L NaCl, 1% NP-40, 1 mmol/L Na_3VO_4, and 1 mmol/L NaF) supplemented with protease and phosphatase inhibitors (Roche, Indianapolis, Indiana). For WB analysis, equal amounts of proteins (10 μg) were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel. Membranes were blocked for 2 hours in blocking buffer (20 mol/L Tris-HCl, 150 mol/L NaCl, 5% nonfat dry milk, and 0.1% Tween 20) at room temperature. Blots were incubated overnight at 4°C with EZH2 1:1000 (Epigentek, Brooklyn, New York). After overnight incubation, membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibody (Sigma, St Louis, Missouri) at room temperature for 1 hour. Proteins were visualized with an automated image documentation system (ChemiDoc, Bio-Rad, Hercules, California) and band density analyzed using Quantity One software (Bio-Rad). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein was used for normalization purposes.

Lysine 27 Trimethylation at Histone 3 Immunoassay

Assessment of H3K27me was conducted using the EpiQuick Immunoassay kit (Epigentek) specific for histone 3 lysine 27 trimethylated following the manufacturer's protocol. In brief, protein samples from whole tissue (200 ng/μL) were added to the wells and incubated according to the protocol. Next, colorimetric analysis was performed using a microplate reader. The amount of methylated protein was obtained by subtracting the methylated protein amount by the total protein amount by the protein input amount and divided by the slope. Two technical replicates were performed for validation purposes.

Immunohistochemistry of H3K27me3 in Human Endometrial and Endometriotic Samples on a TMA

A total of 164 formalin-fixed, paraffin-embedded endometrium and endometriosis tissues were used to construct a TMA at Moffitt Cancer Center.[16] All the biopsies were evaluated by a pathologist to confirm the diagnosis of endometriosis. The total number of cores in the TMA were 34 peritoneal endometriosis, 29 ovarian endometriosis, 16 fallopian endometriosis, 7 gastrointestinal (GI) endometriosis, 4 skin endometriosis, 38 secretory phase endometrium, 14 control proliferative phase endometrium, and 22 eutopic endometrium of women with endometriosis. Control endometria were obtained from patients with other gynecological conditions different to endometriosis. The average age of the patients and controls was 35 and 42 years, respectively.

Immunostaining of H3K27me3 was conducted following standard protocols. Briefly, after paraffin removal and dehydration, antigen unmasking was performed. Peroxidase blocking

Table 1. Demographic Characteristics of Study Patients Analyzed by ELISA.

Pt ID	Diagnosis	Age	ASRM Stage	Tissue	Reg Cycle	Phase
268	Endometriosis	35	III-IV	Ovarian endometriosis	Yes	Secretory
822	Endometriosis	30	I-II	Superficial peritoneal lesion	Yes	Secretory
854	Endometriosis	28	III-IV	Ovarian endometriosis	Yes	Secretory
866	Endometriosis	32	I-II	Superficial peritoneal lesion	Yes	Proliferative
901	Endometriosis	44	I-II	Superficial peritoneal lesion	Yes	Proliferative
1018	Endometriosis	16	I-II	Cul de sac lesion	Yes	Proliferative
1025	Endometriosis	37	III-IV	Ovarian lesion	Yes	Proliferative
1056	Endometriosis	48	I-II	Ovarian lesion	Yes	Secretory
1066	Endometriosis	22	I-II	Superficial peritoneal lesion	No	Proliferative
1068	Endometriosis	17	III-IV	Deep peritoneal lesion	No	Secretory
1080	Endometriosis	20	I-II	Superficial peritoneal lesion	Yes	Proliferative
1084	Endometriosis	42	I-II	Superficial peritoneal lesion	Yes	Secretory
1097	Endometriosis	18	I-II	Superficial peritoneal lesion	Yes	Secretory
1125	Endometriosis	47	III-IV	Fallopian tube endometriosis	Yes	Secretory
805	Endometriosis	25	I-II	Endometrium	Yes	Proliferative
815	Endometriosis	28	I-II	Endometrium	Yes	Proliferative
833	Endometriosis	41	I-II	Endometrium	Yes	Proliferative
834	Endometriosis	36	I-II	Endometrium	Yes	Proliferative
839	Endometriosis	30	I-II	Endometrium	Yes	Proliferative
889	Endometriosis	28	I-II	Endometrium	Yes	Secretory
904	Endometriosis	38	I-II	Endometrium	Yes	Secretory
907	Endometriosis	36	I-II	Endometrium	Yes	Proliferative
920	Endometriosis	32	I-II	Endometrium	Yes	Secretory
924	Endometriosis	38	I-II	Endometrium	Yes	Secretory
947	Endometriosis	22	I-II	Endometrium	Yes	Proliferative
964	Endometriosis	28	III-IV	Endometrium	Yes	Secretory
968	Endometriosis	17	I-II	Endometrium	Yes	Unknown
974	Endometriosis	41	I-II	Endometrium	Yes	Secretory
980	Endometriosis	32	III-IV	Endometrium	Yes	Proliferative
987	Endometriosis	34	I-II	Endometrium	No	Secretory
994	Endometriosis	34	I-II	Endometrium	Yes	Secretory
804	1ry Dysmenorrhea	32		Endometrium	Yes	Secretory
821	Spontaneous Miscarriages	29		Endometrium	Yes	Secretory
829	Spontaneous Miscarriage	42		Endometrium	Yes	Proliferative
905	Endometriosis	38		Endometrium	Yes	Secretory
954	Fibroids	37		Endometrium	Yes	Proliferative
963	CPP	44		Endometrium	Yes	Secretory
966	Fibroids	45		Endometrium	Yes	Proliferative
975	Fibroids	43		Endometrium	Yes	Secretory
983	Fibroids	32		Endometrium	Yes	Proliferative
986	Metrorrhagia	35		Endometrium	No	Unknown
991	Fibroids	47		Endometrium	Yes	Secretory
998	Fibroids	47		Endometrium	Yes	Proliferative
1019	Ovarian tumor	43		Endometrium	No	Unknown
1058	CPP	27		Endometrium	Yes	Proliferative
1095	1ry Dysmenorrhea	21		Endometrium	Yes	Secretory

Abbreviations: ASRM, American Society for Reproductive Medicine; ELISA, enzyme-linked immunosorbent assay.

was conducted and the TMA was incubated with the primary antibody H3K27me3 (1:100) (Epigentek) at 4°C overnight. To amplify and detect the primary antibody signal, we used the labeled streptavidin binding method following the manufacturer's protocol (DAKO, Carpinteria, California). For staining and counterstaining of the TMA, DAB and hematoxylin (Fischer Scientific, Waltham, Massachusetts) were used, respectively. The slide was mounted and visualized at 20×, 40×, and 100× magnification on an inverted microscope with an Olympus 35 mm camera (Nikon, Japan). The Image Scope Software was used to select the areas for analysis (eg, glands and stroma). The Aperio Immunostaining Analysis System available through the Imaging Core from Moffitt Cancer Center was used to evaluate the intensity of H3K27me3 in the endometrial and endometriotic tissues on the TMA. Data were analyzed as percentage of positive nuclei and HistoScore in glands and stroma. HistoScore is obtained from the formula (3 × percentage of strongly staining nuclei) + (2 × percentage of moderately staining nuclei) + (percentage of weakly staining nuclei), giving a range of 0 to 300.

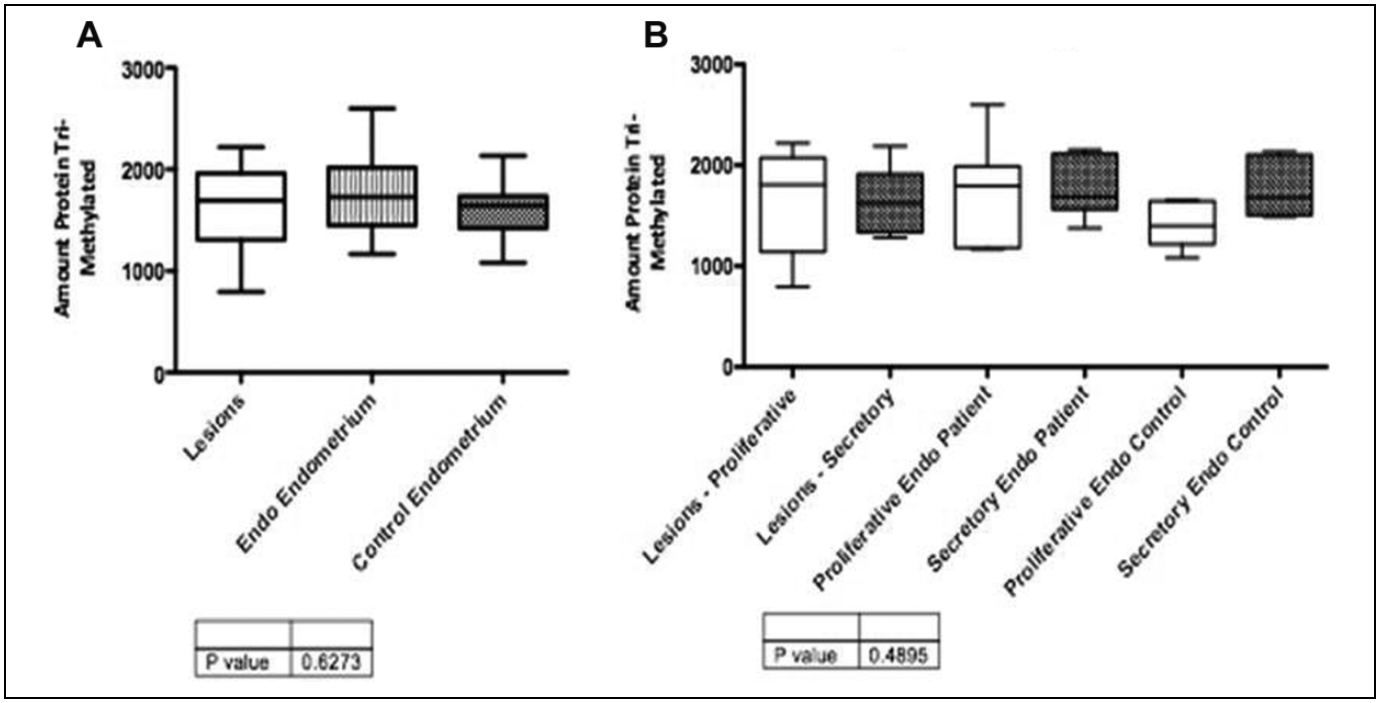

Figure 1. Trimethylation levels in histone 3 lysine (K) residue 27 (H3KK27me3) in endometriosis and endometrium from patients and controls. H3K27me3 levels were determined using the EpiQuick Tri-methylation Assay Kits (Epigentek, Brooklyn, New York) following the manufacturer's protocols. The amount of methylated protein in nanograms per milligrams of total protein was obtained dividing the methylated protein amount and the total protein input amount divided by the slope. Average total trimethylation levels at H3K27me3 were not significantly different ($P = .6273$) in endometriotic lesions as compared to eutopic endometrium from patients and from controls. Endometriotic lesions (n = 14), endometrium from women with endometriosis (n = 17), and endometrium from controls (n = 15).

Statistical Analyses

Nonparametric statistics analysis of variance and Dunn Multiple Comparison posttest were performed using GraphPad Prism 4.0 (GraphPad Software Inc, La Jolla, California) to determine statistical significance between the study groups. Statistical significance was set at $P < .05$.

Results

Levels of H3K27me3 in Endometriotic and Endometrial Tissues Measured by Immunoassay

As shown in Figure 1A, the average total levels of H3K27me3 were not significantly different ($P = .6273$) in endometriotic lesions compared to eutopic endometrium from patients and from controls. No significant differences by menstrual cycle phase were found either ($P = .4895$) between groups, but levels tended to be higher in secretory endometria (Figure 1B).

Percentage of H3K27me3 Positive Nuclei in Endometriotic and Endometrial Tissues on a TMA

The percentage of H3K27me3 (H3K27me3%) positive nuclei was analyzed in different lesion types (ovarian, peritoneal, fallopian, skin, and gastrointestinal [GI (cecum and appendix)]), endometrium from cases, and endometrium from controls on a TMA. Nuclear immunostaining specific for H3K27me3 was detected in both glands and stroma in lesions and endometria. Figure 2 shows representative pictures of the stained TMA showing different levels of staining intensity. No significant differences in percentage of positive nuclei were observed among lesion type in either glands or stroma. Significantly higher levels of H3K27me3% positive nuclei were observed in stroma of lesions and secretory endometrium from controls compared to proliferative endometrium from cases (Figure 3). Similar results were observed for glands; however, levels of H3K27me3 in lesions were not significantly different compared to proliferative endometrium from cases.

Based on the HistoScore, the average intensity of the H3K27me3-specific immunostaining of glands was significantly higher in secretory endometrium compared to proliferative endometrium from both cases and controls. However, no significant differences were found between lesions and control tissues (Figure 4). In stroma, the average intensity of H3K27me3 was not significantly different between lesions and control tissues. Also, we did not observe significant differences in the proportion of tissues showing strong nuclear staining of H3K27me3 in $\geq$50% of nuclei between groups (data not shown).

Progesterone Increases Expression of EZH2 in Endometriotic Epithelial Cells (12Z)

The EZH2 expression profile was analyzed in an endometriotic cell line (12Z) and a nonendometriotic epithelial cell line

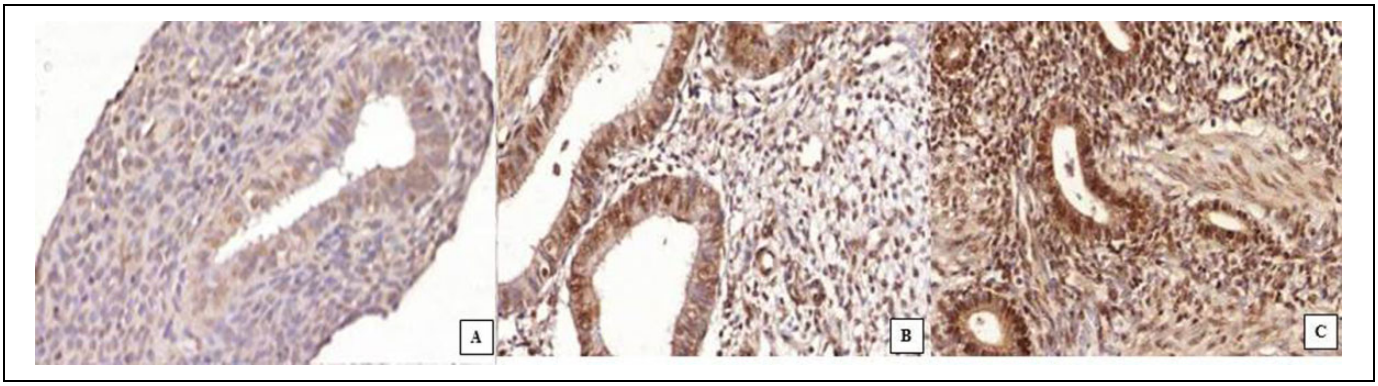

Figure 2. Representative images (×40) of trimethylation of lysine residue 27 at histone 3 (H3K27me3) showing different staining intensities in glands and stroma. Weak (A), moderate (B), and strong intensity (C) of H3K27me3, respectively, are shown. (The color version of this figure is available in the online version at http://rs.sagepub.com/.)

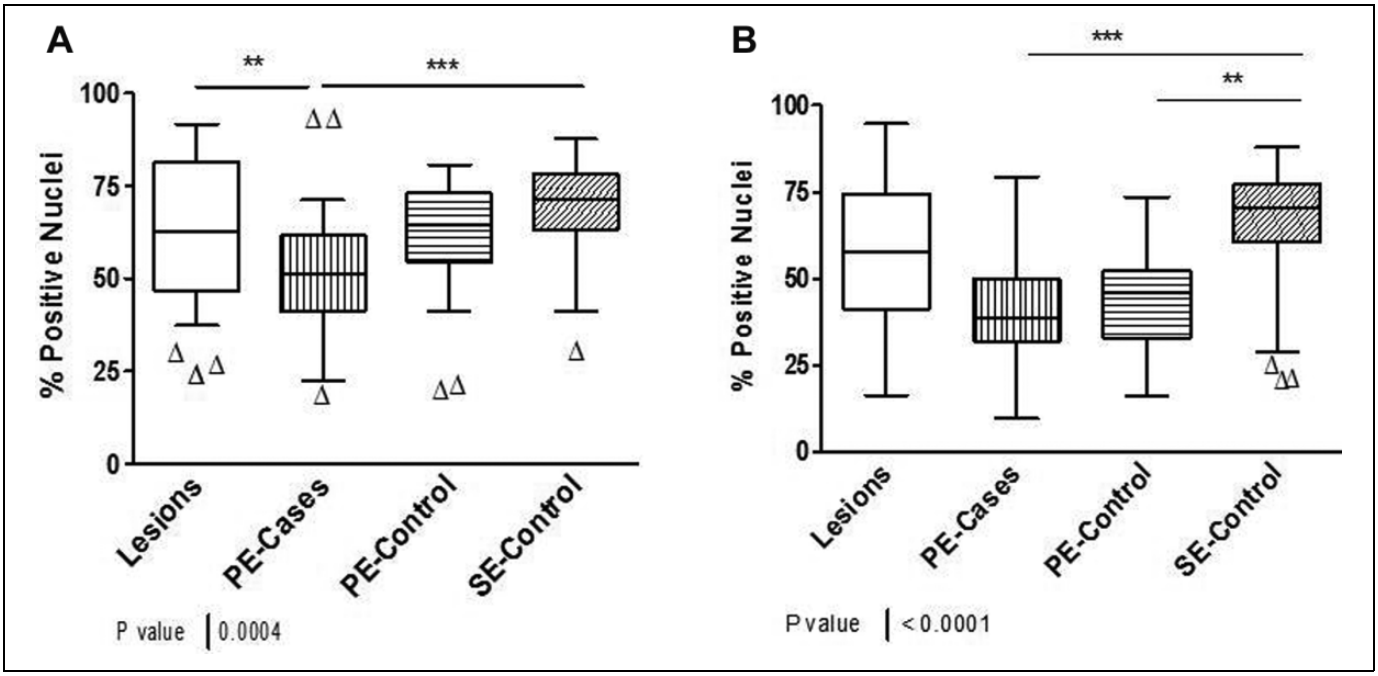

Figure 3. Intensity of Trimethylation of lysine residue 27 at histone 3 (H3K27me3) nuclear immunostaining in endometrial and endometriotic samples. A, H3K27me3 nuclear immunostaining in stroma. A total of 147 of 164 formalin-fixed paraffin-embedded endometrial and endometriotic tissues on a tissue microarray were analysed by immunohistochemistry (IHC). B, H3K27me3 nuclear immunostaining in glands. A total of 130 of 164 formalin-fixed paraffin-embedded endometrial and endometriotic tissues on a tissue microarray were analyzed by IHC. Using the Image Scope Program and Aperio Analysis, the immunostaining intensity of H3K27me3 in both compartments (ie, stroma and glands) was evaluated. One-way analysis of variance (ANOVA) was carried out to compare percentage of positive nuclei in endometriotic lesions, proliferative endometrium from patients (cases), and proliferative and secretory endometrium from controls, respectively.

(EEC/MCF7). Western blot analysis showed that under basal conditions (no treatment), the endometriotic epithelial cells but not the nonendometriotic epithelial cells expressed EZH2. Also, we observed that EZH2 expression was upregulated by progesterone treatment (Figure 5) only in 12Z cells.

Discussion

Epigenetic mechanisms are emerging as important players in the pathophysiology of endometriosis; however, data are limited and the role of histone methylation in endometriosis is not well understood.[2,21,22] It is well known that promoter regions of endometriosis candidate genes such as *HOXA10*, *CDH1*, and *PGR* are hypermethylated contributing to defects in uterine receptivity, invasiveness of endometrial cells, and progesterone resistance, respectively.[23] DNA methylation is known to work together with histone modifications to regulate gene expression. H3K27me3 is a repressive histone mark shown to be closely related to CpG-islands in promoter regions, suggesting a link between histone methylation and DNA methylation.[24] H3K27me3 plays roles during embryogenesis, cell decidualization, tissue regeneration, and pathological states such as

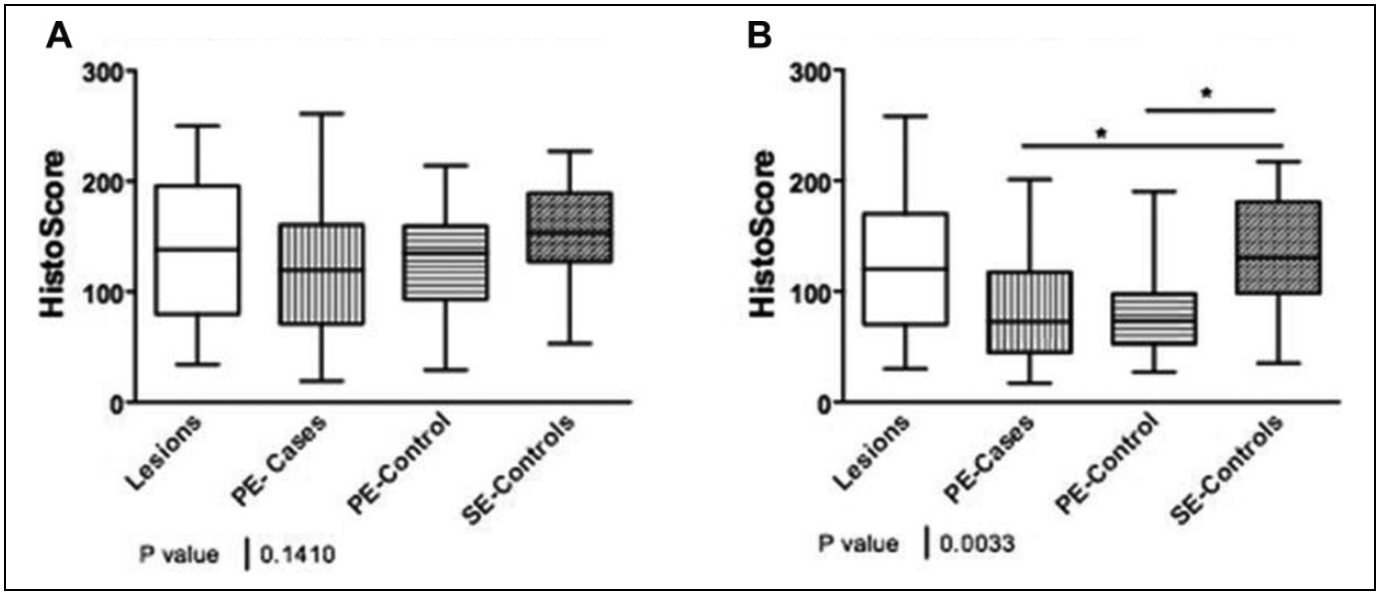

Figure 4. Immunostaining intensity (HistoScore) of H3K27me3 in endometrial and endometriotic tissues. A total 147 of 164 formalin-fixed paraffin-embedded endometrial and endometriotic tissues on a tissue microarray were analysed by immunohistochemistry (IHC). HistoScore for all types of lesions was shown in (A) and (B). Intensities staining for lesions taking together as a group were showed for stroma (A) and glands (B). One-way analysis of variance (ANOVA) was carried out among endometriotic lesion locations and the proliferative endometrium from patients and proliferative and secretory endometrium from controls. Analysis was conducted separately for stroma and glands.

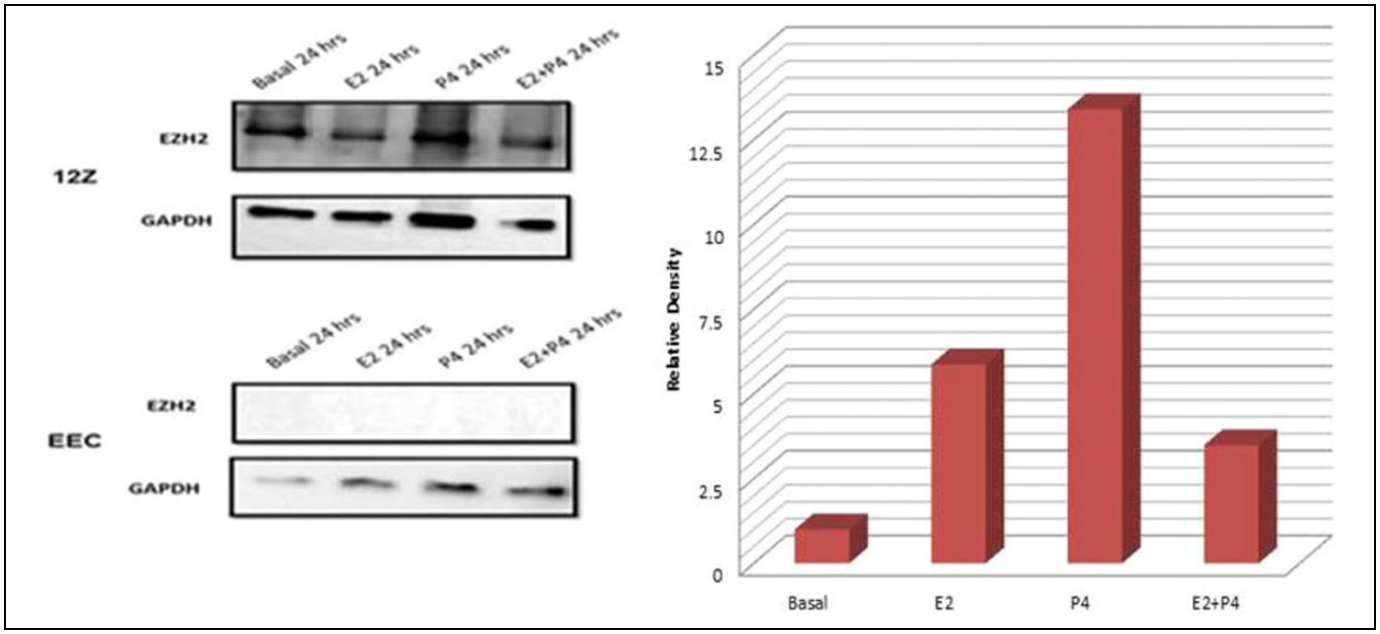

Figure 5. Expression of enhancer of zeste homolog 2 (EZH2) on epithelial endometriotic cells (12Z) and nonendometriotic epithelial cells (EEC/MCF7) with ovarian steroid hormones. EZH2 HMT for H3K27me3 was detected at ≈ 75 to 100 kDa. Total protein of hormone-treated cells (10 μg/μL) was extracted and subjected to Western blot using EZH2 polyclonal antibody. Cells were treated with estrogen (E2), progesterone (P4), and E2/P4 at 24 hours. The concentration of E2 used was 1×10^{-8}. P4 concentration used was 1×10^{-7}. Internal control was GAPDH ≈ 37 kDa. Relative densitometry is shown for 3 independent experiments for the 24-hour time point, optical density (OD) units were normalized against GAPDH and presented relative to basal.

cancer.[25–30] However, the levels of and potential involvement of histone marks in gene expression changes leading to endometriosis are still unknown. This study shows for the first time that endometriotic lesions are characterized by high levels of H3K27me3, which may have pathophysiological and also therapeutic implications.

Although immunoassay results did not show differences in the levels of H3K27me3 in lesions compared to control tissues (endometria from patients and controls), analysis was done on samples representing whole tissues, with varying stromal versus gland ratios. Also, we analyzed a mixture of different lesion types of tissues and clinical presentations. No significant differences were found based on the menstrual cycle phase, but H3K27me3 levels in the secretory phase tended to be higher in eutopic endometrium from patients and controls. This suggests that levels of this histone mark are marginally regulated by progesterone. Previous studies in our laboratory showed that methylation levels of H3K27 were significantly higher in endometriotic lesions and endometrium from patients compared to endometrium of controls, using an immunoassay that does not distinguish among mono-, di-, and trimethylation.[17] The molecular roles of H3K27me1, H3K27me2, and H3K27me3 are known to be different; therefore, it is important to elucidate their respective levels in endometriosis tissues and also to take into account cellular compartment (stroma vs glands). In order to do so and to validate the results obtained with the immunoassay analysis, we analyzed the levels of H3K27me3 in human endometriotic and endometrial tissues on a TMA by IHC.

Based on the percentage of positive nuclei, the levels of H3K27me3 in the glands and stroma of endometriotic lesions (ovarian, peritoneal, and fallopian) were higher than in eutopic endometrium of patients, and this difference reached statistical significance in stroma. In glands, H3K27me3 levels were significantly higher when fallopian tube lesions were excluded from the analysis, which indicates that there are differences based on tissue localization/lesion type. The high interindividual variability observed in the lesions is not expected to be different due to menstrual phase based on the immunoassay results (Figure 1); rather this variability may be related to clinical differences (eg, severity, years with disease, symptoms). The differences observed in H3K27me3 levels in endometrium from patients measured by immunoassay versus IHC can be explained by the fact that the immunoassay included samples in both proliferative and secretory phases, while endometrium from patients in the TMA was only in the proliferative phase. Not only was the percentage of positive nuclei higher in lesions and secretory endometrium from controls compared to proliferative endometrium but also the staining intensity was stronger in these tissues based on HistoScore analysis. These data suggest a potential effect of progesterone in the setting of H3K27me3, perhaps via increases in the expression of EZH2, which we studied next.

Western blot analysis demonstrated that expression of EZH2 by the endometriotic epithelial cell line 12Z was higher at the basal level compared to a control cell line. Protein levels of EZH2 increased in the presence of progesterone only in the endometriotic cells, and none of the ovarian steroid hormone treatments induced expression of EZH2 in the control cell line. These results suggest that EZH2 may be a potential specific target for therapy in endometriosis. Our study is the first to show that the expression of EZH2 is regulated by progesterone, which is in accord with the higher levels of H3K27me3 observed in secretory endometrium. Additional studies are warranted to elucidate a potential role of progesterone in the expression of EZH2 and thus setting of the H3K27me3 histone mark in the context of endometriosis.

In primary human epithelial ovarian cancer cells, EZH2 is overexpressed and its expression correlates with higher expression levels of Ki67, which is a marker of increased proliferation.[13] Different hormone-dependent diseases, including breast, prostate, and endometrial cancers, are characterized by aberrant expression of EZH2 and H3K27me3.[31] H3K27me3 modulates gene expression in different cancer types and processes such as inflammation, migration and metastasis, proliferation, and angiogenesis,[32–37] and these processes were blocked by EZH2 inhibition resulting in reduction of H3K27me3.[38,39] Chromatin (Ch) Immunoprecipitation (IP) - bisulfite sequencing (ChIP-bisulfite-seq) studies demonstrated that CpG islands in promoter regions of cancer cells are characterized by high H3K27me3 levels compared to normal cells[40] and that occupancy of EZH2/H3K27me3 in gene loci is associated with downregulation of genes that regulate stem cells, apoptosis, and cell growth, respectively.[41,42] Trimethylation of H3K27 has been shown to be involved in other normal physiology as well as pathological processes. This histone mark blocks the transition from a proliferative to a decidual phenotype in the endometrium,[28] suppresses the expression of developmental genes such as the HOX genes,[43] and causes COX-2 gene repression and decreases the levels of PGE2.[44] All these cellular processes have been shown to be involved in the pathophysiology of endometriosis, which supports future investigations on the role of the H3K27me3 histone mark in this disease.

The strengths of this study are that we used an endometriosis focused-TMA to assess the nuclear immunostaining of H3K27me3 in different tissue types (eg, lesions, eutopic endometrium from cases, and controls) and cellular compartments (stroma and gland), but more studies are necessary to uncover potential differences based on disease severity or clinical presentation, including assessment of this histone mark in deeply infiltrating lesions. Despite limitations of this study, including the small sample size of the immunoassay, as well as heterogeneity in the cellular composition of the lesions and menstrual cycle phase, these data have important clinical implications. H3K27me3 may be a potential biomarker for endometriosis (its levels were significantly higher in endometria from controls versus endometria from patients, in both glands and stroma); also, EZH2, the enzyme that catalyzes trimethylation of H3K27, may be a novel therapeutic target in endometriosis based on our results showing increased expression of this enzyme in endometriotic cells. Finally, this epigenetic mark may be an important mechanism for regulation of gene expression in this disease. Ongoing studies in our laboratory include

assessment of levels of EZH2 and other HMTs in tissues, and ChIP analysis to profile the H3K27 methylation status near or within the promoter region of candidate genes for endometriosis.

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Authors' Note

This study was carried out at Ponce Health Sciences University—School of Medicine and Ponce Research Institute, Ponce, PR, USA.

Declaration of Conflicting Interests

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References

1. Giudice LC.Clinical practice. Endometriosis. *N Engl J Med*. 2010;362(25):2389-2398.
2. Guo SW. Epigenetics of endometriosis. *Mol Hum Reprod*. 2009; 15(10):587-607.
3. Zhao M, Tang Q, Wu W, Xia Y, Chen D, Wang X. miR-20a contributes to endometriosis by regulating NTN4 expression. *Mol Biol Reprod*. 2014;41(9):5793-5797.
4. Burney O, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril*. 2012;98(3):511-519.
5. Bulun SE.Mechanisms of Disease: Endometriosis. *N Engl J Med*. 2009;360(3):268-279.
6. Munro SK, Farquhar CM, Mitchell MD, Ponnampalam AP. Epigenetic regulation of the endometrium during the menstrual cycle. *Mol Hum Reprod*. 2010;16(5):297-310.
7. Forte A, Cipollaro M, Galderisi U. Genetic, epigenetic and stem cell alterations in endometriosis: new insights and potential therapeutic perspectives. *Clin Sci*. 2014;126(2):123-138.
8. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011;21(3):381-395.
9. Tang H, An S, Zhen H, Chen F. Characterization of combinatorial histone modifications on lineage-affiliated genes during hematopoietic stem cell myeloid commitment. *Acta Biochem Biophys Sin (Shangai)*. 2014;46(10):894-901.
10. Zhang X, Wen H, Shi X. Lysine methylation: beyond histones. *Acta Biochem Biophys Sin*. 2012;44(1):14-27.
11. Kim J, Kim H. Association between endometriosis and polymorphisms in insulin-like growth factor binding protein genes in Korean women. *Eur J Obstet Gynecol Reprod Biol*. 2012; 162(1):96-101.
12. Benoit YD, Laursen KB, Witherspoon MS, Lipkin SM, Gudas LJ. Inhibition of PRC2 histone methyltransferase activity increases TRAIL-mediated apoptosis sensitivity in human colon cancer. *J Cell Physiol*. 2013;228(4):764-772.
13. Li H, Cai Q, Godwin AK, Zhang R. Enhancer of zeste homolog 2 (EZH2) promotes the proliferation and invasion of epithelial ovarian cancer cells. *Mol Cancer Res*. 2010;8(12):1610-1618.
14. Rogenhofer S, Kahl P, Mertens C, et al. Global histone H3 lysine 27 (H3K27me) methylation levels and their prognostic relevance in renal cell carcinoma. *BJU Int*. 2012;109(3):459-465.
15. Venneti S, Santi M, Felicella MM, et al. A sensitive and specific histopathologic prognostic marker for H3F3A K27 M mutant pediatric glioblastomas. *Acta Neuropathol*. 2014;128(5):743-753.
16. Colon-Diaz M, Baez-Vega P, Garcia M, et al. HDAC 1 and HDAC 2 are differentially expressed in endometriosis. *Reprod Sci*. 2012;19(5):483-492.
17. Monteiro J, Colon-Diaz M, Garcia M, et al. Endometriosis is chracterized by a distinct pattern of histone 3 and histone 4 lysine modifications. *Reprod Sci*. 2014;21(3):305-318.
18. Ding D, Liu X, Guo SW. Overexpression of lysine-specific demethylases 1 in ovarian endometriomas and its inhibition reduces cellular proliferation, cell cycle progression, and invasiveness. *Fertli Steril*. 2014;101(3):740-749.
19. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Am J Obstet Gynecol*. 1975;122(2):262-263.
20. Zeitvogel A, Baumann R, Starzinski-Powitz A. Identification of an invasive, N-cadherin-expressing epithelial cell type in endometriosis using a new cell culture model. *Am J Pathol*. 2001; 159(5):1839-1852.
21. Kim J, Taylor H, Lu Z, et al. Altered expression of HOXA10 in endometriosis: potential role in decidualization. *Mol Hum Reprod*. 2007;13(5):323-332.
22. Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor B (PR-B) in endometriosis. *Epigenetics*. 2006;1(2):106-111.
23. Guo SW. The endometrial epigenome and its response to steroid hormones. *Mol Cell Endocrinol*. 2012;358(2):185-189.
24. Ku M, Koche R, Cowan RE, et al. Genomewide analysis of PRC1 and PRC2 occupancy identify two classes of bivalent domains. *PLoS Genet*. 2008;4(10):e1000242.
25. Xu CR, Li LC, Donahue G, et al. Dynamics of genomic H3K27me3 domains and role of EZH2 during pancreatic endocrine specification. *EMBO J*. 2014;33(19):2157-2170.
26. Shpargel KB, Starmer J, Yee D, Pohlers M, Magnuson T. KDM6 demethylase independent loss of histone 3 lysine 27 trimethylation during Early Embryonic Development. *PLoS Genet*. 2014; 10(8):e1004507.
27. Bogliotti YS, Ross PJ. Mechanisms of histone H3 lysine 27 trimethylation remodeling during early mammalian development. *Epigenetics*. 2012;7(9):976-981.

28. Grimaldi G, Christian M, Steel JH, Henriet P, Poutanen M, Brosens JJ. Down-regulation of the histone methyltransferase EZH2 contributes to the epigenetic programming of decidualizing human endometrial stromal cells. *Mol Endocrinol J*. 2011; 25(11):1892-1903.
29. Felician G, Collesi C, Lusic M, et al. Epigenetic modification at Notch Receptor promotes blunts efficacy of inducing Notch Pathway reactivation after myocardial infarction. *Circ Res*. 2014; 115(7):636-649.
30. Yamaguchi H, Hung MC. Regulation and Role of EZH2 in Cancer. *Cancer Res Treat*. 2014;46(3):209-222.
31. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nature*. 2012;13(5):343-357.
32. Chinaranagari S, Sharma P, Chaudhary J. E EZH2 dependent H3K27me3 is involved in epigenetic silencing of ID4 in prostate cancer. *Oncotarget*. 2014;5(16):7172-7182.
33. Tan D, Tan S, Zhang J, et al. Histone Trimethylation of the p53gene by expression of a constitutively active prolactin receptor in prostate cancer cells. *Chin J Physiol*. 2013;56(5):282-290.
34. Lu C, Han HD, Mangala LS, et al. Regulation of tumor angiogenesis by EZH2. *Cancer Cell*. 2010;18(2):185-197.
35. Cao Q, Yu J, Dhanasekaran SM, et al. Repression of E-cadherin by the Polycomb Group Protein EZH2 in Cancer. *Oncogene*. 2008;27(58):7274-7284.
36. Xu C, Hou Z, Zhan P, et al. EZH2 promotes tumor cell migration and invasion via epigenetic repression of E-cadherin in renal cell carcinoma[published online February 25, 2014]. *BJU Int*. 2014.
37. Liu L, Xu Z, Zhong L, et al. EZH2 regulates cancer cell migration through repressing TIMP-3 in non-small cell lung cancer. *Med Oncol*. 2013;30(4):713.
38. Au SL, Wong CC, Lee JM, Wong CM, Ng IO. EZH2-Mediated H3K27me3 is involved in epigenetic repression of deleted in liver cancer 1 in human cancers. *Plos One*. 2013;8(6):e68226.
39. Gao SB, Zheng QF, Xu B, et al. EZH2 represses target genes through H3K27-dependent and H3K27-independent mechanisms in hepatocellular carcinoma. *Am Assoc Cancer Res*. 2014;12(10): 1388-1397.
40. Gao F, Ji G, Gao Z, et al. Direct ChIP-bisulfite sequencing reveals a role of H3K27me3 mediating aberrant hypermethylation of promoter CpG islands in cancer cells. *Cell Biochem Biophys*. 2014; 103(2-3):204-210.
41. Li H, Bitler B, Vathipadiekal V, et al. ALDH1A1 is a novel EZH2 target gene in epithelial ovarian cancer identified by genome-wide approaches. *Cancer Prev Res*. 2012;5(3):484-491.
42. Fu Y, Chen J, Pang B, Li C, Zhao J, Shen K. EZH2-induced H3K27me3 is associated with epigeentic repression of the ARH1 tumor suppressor gene in ovarian cancer. *Cell Biochem Biphys*. 2015;71(1):105-112.
43. Lan F, Bayliss P, Rinn JL, et al. A histone H3 lysine 27 demethylase regulates animal posterior development. *Nature*. 2007; 449(7163):689-694.
44. Coward WR, Feghali-Bostwick CA, Jenkins G, Knox AJ, Pang L. A central role for G9a and EZH2 in the epigenetic silencing of cyclooxygenase-2 in idiopathic pulmonary fibrosis. *FASEB J*. 2014;28(7):3183-3196.