Original Article
Frequent SOCS3 and 3OST2 promoter methylation and their epigenetic regulation in endometrial carcinoma

Haiyan Chen1,2*, Cuijuan Zhang1*, Yan Sheng1, Shuzhe Yao1, Zhiyan Liu1, Cheng Zhang1, Tingguo Zhang1

1Institute of Pathology and Pathophysiology, Shandong University School of Medicine, Jinan 250012, P R China; 2Department of Pathology, Shandong Provincial Chest Hospital, Jinan 250012, P R China. *Equal contributors.

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Abstract: DNA methylation has been considered as an important means of early diagnosis of cancer, which cooperates with histone modifications, playing a crucial role in silencing tumor suppressor genes (TSGs). However, how TSGs are regulated by these epigenetic mechanisms in cancer remains unknown. In this study, we first evaluated 7 TSGs methylation in the early diagnosis of endometrial carcinoma (EC), and then explored the epigenetic mechanisms of their transcriptional regulation. The results showed that SOCS3 and 3OST2 were the most frequently methylated genes in EC (88.3% and 78.3%, respectively), and 3OST2 was correlated with younger patients (< 57 years, P = 0.030) and well-differentiated EC (P = 0.026). Unlike 3OST2, SOCS3 methylation occurred even in complex hyperplasia (53.3%) and atypical hyperplasia (54.2%). 5-aza-2’-deoxycytidine (5-Aza-CdR) or trichostatin A (TSA) alone could partially reverse SOCS3 and 3OST2 methylation, and their combination completely reversed the methylation of both genes. In addition, UHRF1 and methylated H3R8 were enriched on both hypermethylated SOCS3 and 3OST2 promoters, but after 5-Aza-CdR or TSA treatment, the UHRF1 and H3R8me2s enrichment was decreased while H3R8me2a enrichment was increased. In conclusion, we demonstrate for the first time that SOCS3 and 3OST2 methylation plays an important role in endometrial carcinogenesis, and could be directly regulated by UHRF1. Moreover, H3R8me2s acts as a repressive mark, while H3R8me2a was correlated with transcriptional activity in EC.

Keywords: Endometrial carcinoma, DNA methylation, histone modifications, tumor suppressor genes, gene expression

Introduction
Endometrial carcinoma (EC) is one of the most common malignancies of the female reproductive tract worldwide. The accumulation of genetic and epigenetic alterations contributes to the transformation of normal endometrial epithelium to EC via the precursor, atypical endometrial hyperplasia. Promoter methylation, which silences the tumor suppressor genes (TSGs), has emerged as a key mechanism in the origin of many cancers [1-3]. Indeed, aberrant DNA methylation appears to be more frequent than genetic alterations in EC. A growing number of studies have demonstrated that tumors of almost any type involve methylation of a group of TSGs, but normal cells do not. Despite the methylation of multiple TSGs being reported in EC, including that of PTEN, p16, APC, RASSF1A, GSTP1, and E-cadherin, and so on [4-7], far fewer methylated TSGs are found in EC than in other tumors such as hepatocellular carcinoma, breast cancer, and the like. To identify more new methylation biomarkers of EC, the present study investigated the methylation status of 7 TSGs (SOCS1, SOCS3, 3OST2, DLC1, hMLH1, hMSH2, and RARβ2) in EC, and then explored the relationship between DNA methylation and transcriptional regulation.

Covalent modification of histone proteins is another transcription-regulating epigenetic phenomenon during disease processes. It is interestingly clear now that DNA methylation is often colocalized with histone marks in gene repression. Recently, a new protein, ubiquitinlike with PHD and ring finger domain 1 (UHRF1), also known as ICBP90/Np95, was reported to recognize both DNA methylation and histone modifications, and links them [8]. In 2008, three different groups simultaneously reported that the SRA domain of UHRF1 can recognize hemi-
methylated DNA by a base-flipping mechanism [9-11]. Subsequently, it was revealed that UHRF1 also recognizes histone H3K9 trimethylation through its tandem Tudor domain. Therefore, UHRF1 is considered one of several critical molecules linking DNA methylation and histone modifications [12].

Among the diverse covalent modifications of histone N-terminal tails, histone methylation that which can interact with DNA methylation plays an important role in tumor development [13, 14]. Histone methylation occurs on both lysine and arginine residues, and it has been identified as an important modification for both transcriptional activation and transcriptional repression. However, in contrast to lysine methylation, the function of arginine methylation in chromatin structure and transcription is unexplored so far. Arginine methylation exists in three different states: monomethylated (me1), symmetrically dimethylated (me2s), or asymmetrically dimethylated (me2a). More recently, H3R2 methylation has been shown to inhibit the binding of UHRF1 to the histone H3 tail (amino acids 1-21), while H3R8 dimethylation by and large did not significantly affect the binding [15], suggesting that H3R8 dimethylation may bind to UHRF1 and work together to regulate gene expression. In this study, to determine the epigenetic transcriptional mechanisms of hypermethylated TSGs in EC and understand the interactions between DNA methylation and histone modifications, we aimed first to explore whether UHRF1 was involved in endometrial carcinogenesis and whether it could directly regulate the expression of the hypermethylated TSGs on their promoters. Next, we investigated whether H3R8 could be monomethylated or dimethylated (me2s or me2a) in transcriptional regulation of the TSGs, and whether the three methylation states would exert similar or opposite effects. In addition, the effects of a DNA methyltransferase (DNMT) inhibitor, 5-aza-2'-deoxycytidine (5-Aza-CdR), and a histone deacetylase (HDAC) inhibitor, trichostatin A (TSA), alone or in combination on UHRF1 and H3R8 methylation as well as TSG methylation and expression were observed.

**Materials and methods**

**Patients and samples**

Paraffin-embedded tissue blocks of normal endometrium (n = 27), simple hyperplasia (n = 25), complex hyperplasia (n = 30), atypical hyperplasia (n = 24), and endometrial adenocarcinoma (n = 60) were obtained from Shandong University Qilu Hospital between 2009 and 2012. We collected the clinical parameters of each patient, namely age, tumor differentiation, depth of myometrial invasion, and lymph node metastasis. All lesions were diagnosed according to the diagnostic criteria.
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defined by the World Health Organization (WHO, 2008). Ethics approval was obtained from the Committee of Ethical Research at Shandong University.

**Cell lines and drugs treatment**

The human endometrial cancer cell line Ishikawa (the European Collection of Cell Cultures, Sigma, UK) was maintained in RPMI-1640 with 10% fetal bovine serum. The Ishikawa cells were treated with 5-Aza-CdR and TSA either alone or in combination. The cells were exposed continuously to 8 μmol/L 5-Aza-CdR for four days or to 300 ng/ml TSA for two days (Sigma, St. Louis, MO, USA). For combined treatment, the cells were initially treated with 8 μmol/L 5-Aza-CdR for two days and subsequently co-treated with 300 ng/mL TSA for an additional two days.

**Methylation-specific PCR (MSP)**

Genomic DNA was extracted from the paraffin blocks and the Ishikawa cells with a Genomic DNA Purification Kit (Qiagen, Hilden, Germany). The extracted DNA (1 µg) underwent bisulfite conversion using a CpGenome DNA Modification kit (Intergen Co., Purchase, NY, USA), according to the manufacturer’s protocol. In brief, 2 μL of modified DNA was PCR-amplified in a total volume of 50 μL. The primer sequences and annealing temperatures are listed in Table 1. The PCR products were separated on 2% agarose gels and visualized with ethidium bromide staining.

**Reverse transcription real-time quantitative PCR (qRT-PCR)**

Total RNA was extracted from the Ishikawa cells treated or untreated with the inhibitors using TriZol (Life Technologies, Rockville, MD, USA). First-strand cDNA was synthesized from 1 µg total RNA with Primer Mix and RT Enzyme Mix (Toyobo, Japan) according to the manufacturer’s instructions. The qRT-PCR was performed in a 20-μL reaction, which included 3 μL cDNA template, 5 μM each of forward and reverse primer, and 10 μL SYBR Green I (Bio-Rad, Hercules, CA, USA). The following primer pairs were used: SOCS3 (forward, 5’-TTCTGCTATCATT-3’; reverse, 5’-TCCTTGAGTGGAGG-3’), and UHRF1 (forward, 5’-CCAGCAGCAGCTCTCATC-3’; reverse, 5’-TCCTTGAGTGGAGG-3’).

**Immunohistochemistry**

The Ishikawa cells were seeded in a six-well plate that contained slides. The inhibitors were added after the cells were adhered. To ensure that the antibody could enter the nucleus, the slides were incubated with 0.5% TritonX-100 for 30 min. The slides were incubated with anti-UHRF1 antibody (1:500; Abcam, Cambridge, UK) at 4°C overnight, followed by incubation with secondary antibody at room temperature for 30 min and visualized using diaminobenzidine. Only distinct nuclear staining was considered as positive. The protein levels of all groups were evaluated by a staining score system based on the percentage of positive cells and staining intensity. At least 1000 cells were counted for each slide. Four semi-quantitative classes were used to describe staining intensity (intensity score: absent, 0; weak, 1; moderate, 2; strong, 3) and the percentage of positive cells was graded as follows: extent score: absent, 0; < 10%, 1; 10-50%, 2; > 50%, 3. By multiplying the intensity score with extent score, a score index was derived, ranging from 0 to 9.

**Western blot analysis**

Acid extraction of histones from cells treated or untreated with inhibitors was performed according to the instructions provided by Upstate Biotechnology. Cellular nuclear protein (50 μg/ lane) was separated by 12% SDS-PAGE and hybridized separately with antibodies specific for UHRF1 (1:500; Active Motif), H3R8me1 (1:200; Active Motif), H3R8me2s (1:200; Novus Biologicals) and H3R8me2a (1:500; Novus Biologicals) at 4°C overnight, followed by incubation with secondary antibody for 1 h at room temperature. After extensive washes, the membranes were visualized with ECL plus™ Western Blot Detection System (Amersham Biosciences, Buckingham, UK). To determine the relative expression level of each sample, the β-actin expression level was measured as the internal control.

**Chromatin immunoprecipitation (ChIP)-qPCR**

In brief, proteins were cross-linked to DNA by adding 1% formaldehyde, and the lysates were
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Table 2. Multiple tumor suppressor genes (TSGs) methylation frequencies in normal endometrium, simple hyperplasia, complex hyperplasia, atypical hyperplasia, and endometrial carcinoma

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal endometrium</th>
<th>Simple hyperplasia</th>
<th>Complex hyperplasia</th>
<th>Atypical hyperplasia</th>
<th>Endometrial carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOCS1</td>
<td>0%</td>
<td>0%</td>
<td>1.000</td>
<td>3.3%</td>
<td>1.000</td>
</tr>
<tr>
<td>SOCS3</td>
<td>14.8%</td>
<td>24%</td>
<td>0.492</td>
<td>53.3%</td>
<td>0.005**</td>
</tr>
<tr>
<td>3OST2</td>
<td>11.1%</td>
<td>16%</td>
<td>0.698</td>
<td>33.3%</td>
<td>0.061</td>
</tr>
<tr>
<td>DLC1</td>
<td>0%</td>
<td>0%</td>
<td>1.000</td>
<td>6.7%</td>
<td>0.492</td>
</tr>
<tr>
<td>hMLH1</td>
<td>3.7%</td>
<td>4.0%</td>
<td>1.000</td>
<td>3.3%</td>
<td>1.000</td>
</tr>
<tr>
<td>hMSH2</td>
<td>0%</td>
<td>0%</td>
<td>1.000</td>
<td>0%</td>
<td>1.000</td>
</tr>
<tr>
<td>RARβ2</td>
<td>14.8%</td>
<td>28.0%</td>
<td>0.317</td>
<td>13.3%</td>
<td>1.000</td>
</tr>
</tbody>
</table>

p value is relative to normal endometrium (χ² tests). *p < 0.05; **p < 0.01.

Figure 1. Methylation analysis of the promoters of 7 tumor suppressor genes (SOCS1, SOCS3, 3OST2, DLC1, hMLH1, hMSH2, and RARβ2) in endometrial carcinoma. The MSP products in the M lanes indicate the presence of methylated alleles, and those in the U lanes indicate the presence of unmethylated alleles.

sonicated to shear DNA to average lengths of approximately 200-1000bp. To reduce nonspecific background, the DNA-protein complexes were pre-cleared by incubation with 80 μL of Protein A agarose beads. Anti-symmetrical dimethyl-H3R8 antibody (5 μg; Novus Biologicals), anti-asymmetrical dimethyl-H3R8 antibody (5 μg; Novus Biologicals), or normal rabbit serum was added and incubated overnight at 4°C. The primers used for the ChIP-qPCR were: SOCS3: (forward, 5’-GGTCACCCACAGCAAGTTTCC-3’; reverse, 5’-TCGCGGATCAGAAAGGTGC-3’); and 3OST2: (forward, 5’-GTGCCACACAGCAAGTTTCC-3’; reverse, 5’-GTTCCAACACAGCAAGTTTCC-3’). The primer used for the ChIP-qPCR was: SOCS3: (forward, 5’-GGTCACCCACAGCAAGTTTCC-3’; reverse, 5’-TCGCGGATCAGAAAGGTGC-3’); and 3OST2: (forward, 5’-GTGCCACACAGCAAGTTTCC-3’; reverse, 5’-GTTCCAACACAGCAAGTTTCC-3’).

Statistical analysis

Statistical analysis was performed using SPSS 16.0 for windows (SPSS Inc, Chicago, IL). Differences of protein expression among groups were tested by one-way analysis of variance (ANOVA). Frequencies of methylation were compared using Chi-square test and Fisher exact test. A value of P < 0.05 was considered statistically significant.

Results

Promoter methylation status of TSGs in a series of primary endometrial lesions

Table 2 lists the methylation rates of the 7 genes in a series of endometrial lesions. Representative examples of the MSP are illustrated in Figure 1. Among the TSGs studied, the most frequently methylated genes in EC were SOCS3 (88.3%) and 3OST2 (78.3%), which were selected for further analysis. Interestingly, high frequencies of SOCS3 methylation were
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also found in complex hyperplasia (53.3%) and atypical hyperplasia (54.2%) as compared with normal endometrium (14.8%) and simple hyperplasia (24%), and there was no difference between the two groups. Significant differences of SOCS3 methylation between complex hyperplasia/atypical hyperplasia and EC were also found (P < 0.001 and P = 0.002, respectively). Unlike SOCS3, 3OST2 was frequently methylated in only the EC group (78.3%) but much less so in the other groups (from 11.1% to 37.5%).

The association between gene methylation and clinicopathological parameters such as age, tumor differentiation, depth of myometrial invasion, and lymph node metastasis was also analyzed (Table 3). 3OST2 methylation was correlated with younger patients (< 57 years, P = 0.030) and well-differentiated EC (P = 0.026), while no correlation between SOCS3 methylation and any clinicopathological parameters was found.

SOCS3 and 3OST2 promoter methylation and mRNA expression in EC cells before and after treatment with 5-Aza-CdR and/or TSA

To demonstrate whether the methylation of the TSGs could directly control their transcription, we investigated the changes in SOCS3 and 3OST2 promoter methylation status and mRNA expression in the Ishikawa cells after treatment with 5-Aza-CdR with MSP and qRT-PCR, respectively. To further elucidate the relationship between DNA methylation and histone modifications, we also treated the Ishikawa cells with TSA alone or initially treated cells with 5-Aza-CdR and then in combination with TSA.

The MSP showed that both SOCS3 and 3OST2 promoters were completely methylated in Ishikawa cells and could be partially reversed by 5-Aza-CdR. Interestingly, TSA alone also could alter the methylation status of the two genes. Moreover, the promoter methylation of both genes was completely reversed by the two inhibitors in combination (Figure 2A, 2B). The qRT-PCR revealed significant elevated mRNA expression of both genes after treatment with TSA (Figure 2C, 2D). Although the fold increase was relatively small (< 2 fold) by 5-Aza-CdR, it was a statistically significant change. Combining 5-Aza-CdR with TSA synergistically increased SOCS3 and 3OST2 mRNA expression in the Ishikawa cells.

UHRF1 expression changes in EC cells after treatment with 5-Aza-CdR and/or TSA

UHRF1, which possesses several domains that can read both DNA methylation and histone methylation, thus, physically linking these two epigenetic marks, is overexpressed in numerous cancers. However, its role in EC has not been investigated. We first performed immunohistochemistry to detect the protein expression of UHRF1 in the Ishikawa cells. The results showed that UHRF1 was highly expressed in the cells (Figure 3) and moderately downregulated by 5-Aza-CdR alone (P = 0.029). TSA exhibited more efficient than 5-Aza-CdR (P = 0.001). After treatment with 5-Aza-CdR combined with TSA, the UHRF1 expression was significantly decreased (P < 0.001).

Next, to quantitatively measure the levels of UHRF1 expression changes after treatment with 5-Aza-CdR and/or TSA, we performed qRT-PCR and western blot assays. The results demonstrated that the mRNA and protein levels of UHRF1 in the Ishikawa cells were very high.
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(Figure 4A-C), and were sharply reduced by treatment with 5-Aza-CdR or TSA (P < 0.001). The combination treatment was more effective than either inhibitor alone, which was consistent with the result of immunohistochemistry.

Functions of three methylation states of histone H3R8 in EC cells

Multiple arginine residues in histone tails, including R2, R8, R17 and R26 in H3 and R3 in
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H4 have been shown to be monomethylated, or symmetrically or asymmetrically dimethylated [16, 17]. In order to understand whether H3R8 could be methylated in Ishikawa cells, we first performed western blot analysis in the whole-cell extract treated or untreated with the inhibitors. The results showed that H3R8me1 and H3R8me2s were present prominently in the untreated cells and were decreased after treatment with 5-Aza-CdR or TSA (Figure 5). On the contrary, the levels of H3R8me2a in the untreated cells were very low and significantly increased only after TSA treatment. There was slight elevation after 5-Aza-CdR treatment, but no statistical significance was found. When combined with the two inhibitors, H3R8me1 and H3R8me2s levels were significantly reduced; concurrently, H3R8me2a levels were significantly increased.

**ChIP-qPCR assays**

To investigate whether UHRF1 and H3R8 methylation were directly involved in the transcriptional regulation of the hypermethylated TSGs, and whether they could be altered by 5-Aza-CdR and/or TSA treatment, we further conducted ChIP assays, followed by ChIP-qPCR. We determined that UHRF1 was recruited to both the SOCS3 and 30ST2 promoters in the Ishikawa cells, and the recruitments were decreased moderately by the 5-Aza-CdR treatment, while the TSA treatment decreased...
recruitments significantly (Figure 6), consistent with the results of the qRT-PCR and western blot, indicating that SOCS3 and 3OST2 expression is directly regulated by UHRF1. Furthermore, the ChIP-qPCR assays also showed that there was enrichment of both H3R8me2s and H3R8me2a binding to the SOCS3 and 3OST2 promoters, but the H3R8me2s enrichment was significantly decreased after exposure to TSA, while that of H3R8me2a was increased accordingly. There was only slight or moderate change of the two marks after 5-Aza-CdR treatment. The 5-Aza-CdR and TSA combination had synergistic effects on the reduction of UHRF1 or H3R8me2s recruitment to the promoters as well as the binding of H3R8me2a to the promoters. As no ChIP-grade H3R8me1 antibody was available, the role of H3R8me1 in regulation of the SOCS3 and 3OST2 genes is yet to be studied.

**Discussion**

EC is subcategorized into type I and II according to pathological characteristics. The molecular mechanisms involved in type I EC appear to be distinct from type II [18]. Type I EC frequently demonstrates microsatellite instability, and genetic (e.g., mutations, gene amplification/deletion) or epigenetic alterations (primarily promoter DNA methylation). By contrast, type II EC exhibits less promoter hypermethylation and more frequent DNA mutations [19, 20]. Based on this literature, 60 type I EC samples were included in this study. The results provided the specific TSG methylation profile for EC and demonstrated that SOCS3 and 3OST2 are the most frequently methylated genes in EC but the frequency is very low in normal endometrial samples.

Suppressor of cytokine signaling (SOCS) proteins are inhibitors of cytokine signaling that function via the JAK/STAT pathway [21]. Among the eight SOCS family members, SOCS1 and 3 are best characterized for their regulation and functions, which are downregulated in several human cancers. Cooper et al. has reported that in leukemia cells, the SOCS1 gene is silenced by DNA hypermethylation, while the loss of SOCS3 expression occurs through a mechanism independent of epigenetic silencing by DNA methylation [22]. However, in our study, SOCS3 but not SOCS1 hypermethylation played an important role in endometrial carcinogenesis. The methylation rate of SOCS3 increased as the lesion grade increased, and although there was a statistically significant difference between the EC group and the other groups, frequent methylation of the SOCS3 promoter were also demonstrated in the complex hyperplasia and atypical hyperplasia samples (53.3% and 54.2%, respectively). This suggested that the DNA methylation process starts in this gene before it expands to involve EC, which is consistent with the findings of Nieminen et al., who pointed out that, contrary to the traditional view, complex hyperplasia both without and with atypia are equally important as precursor lesions of EC [23]. The 3OST2 gene, which encodes an O-sulfotransferase responsible for the final modification step of heparin sulfate

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Figure 6. ChIP-qPCR assays for UHRF1, H3R8me2s and H3R8me2a on (A) SOCS3 and (B) 3OST2 promoters. Cross-linked chromatin from Ishikawa cells treated or untreated with 5-Aza-CdR and/or TSA underwent immunoprecipitation using control IgG, anti-UHRF1, anti-H3R8me2s or anti-H3R8me2a antibody, and the immunoprecipitated DNA was analyzed by qPCR using primers specific for 3OST2 and SOCS3. Shown is the enrichment, relative to input obtained in each ChIP sample. *P < 0.05 versus controls; **P < 0.01 versus controls.
proteoglycans (HSPGs) that are important in the field of migration, cell growth and adhesion [24], also undergoes frequent promoter methylation in EC (78.3%). However, different from SOCS3, 3OST2 was frequently methylated in only the EC group but much less so in the other groups, including the complex hyperplasia group and atypical hyperplasia group.

Taken together, our data indicate that SOCS3 methylation is a very early event in EC development, even during the precancerous stage, just as we and others have demonstrated the presence of some TSGs methylation in morphologically normal cells adjacent to cancer tissues [25-27]. During endometrial carcinogenesis, certain TSGs such as SOCS3 are methylated at the early stage, while others such as 3OST2 may be methylated at the later stage, a reflection that each TSG may function differently and that the accumulation of these TSG silencing will promote the multistep process of cancer. As for the early diagnostic value of the studied TSGs in differentiating EC from atypical hyperplasia, we consider 3OST2 methylation better than SOCS3 methylation, which occurred even in complex hyperplasia. Although there was a significant difference in DLC1 methylation between the EC group and the other groups, its low methylation frequency limits its diagnostic value in clinics. In addition, the present study assessed the association between SOCS3 or 3OST2 methylation and clinical characteristics. 3OST2 methylation was found to be associated with well-differentiated EC and younger patients. It should be noted that as well-differentiated EC often occurs in younger patients, the correlation between 3OST2 methylation and younger patients may be an accompanied phenomenon.

Unlike genetic alterations, epigenetic changes are reversible. It was generally believed that the expression of methylated genes can be restored only by DNMT inhibitors, and that HDAC inhibitors only activate the transcription of unmethylated genes [28-30]. In the present study, we used an endometrial cell line (another cell line, a hepatocellular carcinoma cell line was also used, data not shown) to observe the effects of these inhibitors. However, our data show that either 5-Aza-CdR or TSA alone was able to partially reverse the TSG promoter methylation, and after treatment with 5-Aza-CdR in combination with TSA, both SOCS3 and 3OST2 promoter methylation were completely reversed, and their expression was synergistically increased, demonstrating the cross-talk between DNA methylation and histone modifications. Recently, Arzenani et al. reported that TSA treatment could reduce global DNA methylation and the DNMT1 protein level and could alter DNMT1 nuclear dynamics and interactions with chromatin [31]; this may explain why TSA could reverse the TSG methylation.

A growing body of evidence has revealed that UHRF1, characterized by reading both DNA methylation and histone modifications, interacts with many histone modulators, including HDAC1, G9a, DNMT3a, DNMT3b, and Tip60 [32-35], and plays a fundamental role in DNA methylation, histone methylation, histone acetylation, cell proliferation, and apoptosis [36-39]. Our results showed that UHRF1 was highly expressed in EC cells, and was downregulated by 5-Aza-CdR or TSA alone. UHRF1 binds to the methylated promoter regions of various TSGs, including p16INK4A and p14ARF, via its SRA domain [35], which is found only in the UHRF family [40]. The present study showed that UHRF1 could bind to both SOCS3 and 3OST2 promoters to directly repress their expression. In addition, H3R8 methylation was involved in the suppression of SOCS3 and 3OST2 genes in EC. Pal et al. reported that H3R8me2s correlated with the repression of the suppressor ST7 in lymphoid cancer cells [41]. However, our results revealed that H3R8me2s was a repressive mark, while H3R8me2a was linked to TSG activation, opposite to the function of H3R8me2s. Although the western blotting results showed that H3R8me1 was present in EC and was inhibited by 5-Aza-CdR and/or TSA, its regulation role of SOCS3 and 3OST2 remains unexplored. In our data, all three methylation states of H3R8 could be changed by 5-Aza-CdR and/or TSA, suggesting that the two inhibitors may affect not only DNMT1 but also protein arginine methyltransferases (PRMTs), although which PRMT is involved needs to be further studied.

In conclusion, we have identified two new methylation biomarkers to warn of EC, and gain valuable insight into not only the role of epigenetic changes in regulation of the methylated TSGs but also the mechanisms of interaction between DNA methylation and histone modifications. We also describe for the first time that H3R8me2s acts as a repressive mark, while
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H3R8me2a was correlated with transcriptional activity in EC.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Tingguo Zhang or Cuijuan Zhang, Institute of Pathology and Pathophysiology, Shandong University School of Medicine, Jinan 250012, P. R. China. Tel: 86-531-88382574; Fax: 86-531-88383168; E-mail: ztguo@sdu.edu.cn (TGZ); E-mail: cuijuan.zhang@sdu.edu.cn (CJZ)

References


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