Progestosterone Receptor B Gene Inactivation and CpG Hypermethylation in Human Uterine Endometrial Cancer

Masahiro Sasaki, Abhipsa Dharia, Bong R. Oh, Yuichiro Tanaka, Sei-ichiro Fujimoto, and Rajvir Dahiya

ABSTRACT

The expressions of two isoforms of human progesterone receptor (PR) are under the control of the two different promoters. Recent studies revealed differences between these isoforms, PRA and PRB, in their expression and function in endometrial cells. Abrupt methylation of normally unmethylated CpG islands has been associated with inactivation of several genes in human cancers. In this study, we investigated the methylation status and the expression of the two different PR isoforms, PRA and PRB, in uterine endometrial carcinoma (UEC) using methylation-specific PCR (MSP), reverse transcription-PCR (RT-PCR), the 5'-rapid amplification of cDNA ends method (5'-RACE), and immunohistochemical staining. The results of RT-PCR and 5'-RACE suggest that only PRB is inactivated, although PRA is activated in all UEC cell lines. Treatment with a demethylating agent, 5-aza-2'-deoxycytidine, restored PRB expression in all cell lines, suggesting that inactivation of this gene is through methylation. By MSP and direct DNA sequencing, PRB was methylated, whereas PRA was unmethylated in all of the cell lines. To determine the methylation status of PRB in UEC patients, we investigated 83 cancerous and 33 normal samples. Sixty-two of 83 cancer samples had only methylated alleles of PRB, although all cancer samples had only unmethylated PRA alleles. Seventy-one of 83 cancer samples were negative for PRB expression. All 62 cancer samples that had only methylated PRB alleles were negative for PRB expression. No significant changes were observed in PRA methylation status or immunohistochemistry positivity in normal and cancer samples. To determine whether de novo methylation of PRB occurred in UEC patients, we studied 32 pairs of cancer and normal samples from the same patient. Twenty of 32 cancer samples had only methylated PRB alleles, although all 32 normal samples had only unmethylated PRB alleles. The loss of unmethylated alleles was well correlated with negativity in immunohistochemical staining for PRB. This is the first report of the selective methylation and the subsequent silencing of PRB in uterine endometrial cancer.

INTRODUCTION

UEC is a common gynecological malignancy of the female urogenital tract, and its incidence is significantly increasing (1). However, the genetic basis of tumorigenesis in this malignancy is not well understood. Progesterones are involved in many regulatory processes in the endometrial epithelium (2). Progesterones protect the endometrium against the hyperplastic effects of estrogen through PRs.

Endometrial cancer induced by hyperestrogenism is suppressed by progesterone (2). This conclusion is based on studies of an association between endometrial cancer and polycystic ovarian disease as well as other hyperestrogenic states. In these diseases, the tumorigenic effect of estrogen is completely reversed by the addition of a progestational agent (3). Progesterone counteracts the growth-stimulatory effects of estrogen by inducing glandular and stromal differentiation of endometrium. Progesterone treatment is effective in decreasing the growth of endometrial tumors, which express PRs. The activity of PR strongly correlates with successful endocrine treatment and patient’s survival (3).

Progesterone effects are mediated via PR. However, several studies have shown that PR regulation may be more complex than suspected previously. The expression of human PR is controlled by two promoters (4). Human PR has two isoforms, A and B, which originate from translational initiation at AUG2 and AUG1, respectively. Both PR isoforms have a hormone-binding domain at the COOH terminus, a DNA-binding domain through which the receptors contact DNA, and two variable-length NH2 termini. Several investigators have suggested that the unique NH2-terminal sequence of the B receptor contain a strong transcriptional activating function (5, 6). There are several reports that observed that the functional activities of PRA and PRB are different in a cell type-, promoter-, or ligand-specific manner (7–9). It is possible that the different mechanisms for the regulation of PR isoforms reflect their expressions in the endometrium.

It has been speculated that PRA and PRB are expressed in the endometrium with equal frequency and have similar function (10). However, recent studies uncovered a difference between PRA and PRB expression and their function in endometrial and breast cancers (11–15). In the endometrium, cyclical effects of estrogen and progesterone are mediated by PR regulation. However, progesterone regulation of PR is not a uniform effect in the uterus (10–15). In glands, the persistence of PRB during the midsecretory phase suggests its significance in glandular secretion. In stroma, the predominance of PRA throughout the cycle suggests its significance in postovulatory events. These results support the view that PRA and PRB mediate distinct pathways of progesterone action in the glandular epithelium and stroma of the human uterus throughout the menstrual cycle (10). Several researchers reported that the PRA:PRB ratio is abnormal in endometrial cancer, leading to a lack of normal progesterone protection against the growth-promoting effects of estrogen (16, 17). The PR gene contains CpG islands in the 5' upstream region (4). CpG islands are areas rich in CG dinucleotides that are found within the promoter region of various genes (17, 18). Abnormal CpG island methylation seems to be a frequent event in most malignancies (19, 20). Hypermethylation of promoter-associated CpG islands has been associated with gene silencing. De novo methylation of promoter-associated CpG islands has been associated with the transcriptional inactivation of genes. Thus, such methylation may be functionally equivalent to an inactivating mutation for the silencing of these genes (20–22). However, no previous evaluation of the CG-enriched region 5' to exon 1 of PR has been reported in normal endometrium or UEC cells.

It is not known whether the effects of methylation on the two different PR promoters lead to equivalent silencing in the levels of PRA and PRB expressions. This question is critical in understanding the complexity of PR expression and regulation in the UEC. In this report, we studied methylation status and the expression of the two isoforms of this gene using several UEC cell lines and cancerous and normal tissues from UEC patients.
MATERIALS AND METHODS

Primary Uterine Endometrial Tumors and Cell Lines. Human endometrial cell lines, Ishikawa, HHUA, HEC-IB, and MFE-296, were maintained in phenol red containing DMEM with 10% FCS (23). The cells were treated with a freshly prepared solution of 5-azaC (Sigma Chemical Co., Santa Cruz, CA). On day 1, a final concentration of 2 μg/ml 5-azaC in PBS was added to the flask. The next day, the medium was changed. On days 3 and 5, the cells were treated with 5-azaC two more times. On day 6, the cells were harvested. Samples were obtained from the Department of Gynecology at the hospital of Hokkaido University. DNA was obtained from 83 cancer and 33 normal uterine samples. The histopathological types of these cancers were as follows: 63 samples, endometrioid cancer; 2 samples, adenosquamous cancer; 5 samples, adenoacanthoma; and 2 samples, clear cell cancer; and 11 samples, unknown type. In addition, we also tested 32 pairs of cancerous and normal endometrial samples from the same UEC patient.

DNA Extraction and Sodium Bisulfite Treatment. DNA was isolated from the samples scraped from paraffin-embedded sections. Microdissections were done from these samples as described previously (23). DNA (~100 ng) was denatured using NaOH and treated with sodium bisulfite for 16 h (Introgen, Purchase, NY) as described previously (23). Modified DNA was resuspended in 50 μl of TE (0.1 M Tris-HCl, 1 mM EDTA) and stored immediately at −20°C.

MSP Conditions. The primers and PCR conditions are summarized in Table 1 and Fig. 1. These primer sequences were chosen for regions containing frequent cytosines (to distinguish unmodified from modified DNA) and CpG pairs near the 3′ end of primers (23). These primer sets are located on the 5′ upper region of each promoter for distinguishing PRA and PRB separately (22). A fragment of DNA to be amplified was intentionally small, because larger fragments are not possible from paraffin blocks. In this examination, we used a breast cancer cell line, MDA-MB-231, as a control for methylated bands, and this cell line was treated with 5-azaC as a control for unmethylated bands.

RNA Isolation, RT-PCR, and 5′RACE. Cells were washed and lysed using a guanidine isothiocyanate solution. Total RNA was isolated by phenol-chloroform extraction according to our previous method (23). RT-PCR kits (Perkin-Elmer Corp.) were used to synthesize cDNA from 1.5 μg of total RNA using random hexamer primers. cDNA synthesis was carried out as suggested by the kit protocol, using murine virus reverse transcriptase. The reverse transcription was carried out for 60 min at 42°C. After an incubation at 99°C for 5 min to inactivate the reverse transcriptase, the entire 20 μl of cDNA were used to amplify regions of PR common to both isoforms and region unique to PRB. β-actin cDNA fragments were also amplified as a positive control (Table 1). Primers for β-actin were chosen specifically to cross two exons in the β-actin gene. In the presence of contaminating genomic DNA, additional larger bands would be amplified. The lack of amplification of any larger bands would indicate that there was no contamination with any genomic DNA. Negative controls without RNA and without reverse transcriptase were also performed.

PRA mRNA cannot be distinguished from PRB by conventional RT-PCR because PRA has no specific sequence to distinguish it from PRB mRNA. A modified 5′RACE method was used for accurate evaluation of each mRNA expression (24). RNA (5 μg) was reverse-transcribed using a custom-designed 24-mer oligonucleotide, RACE-REV, 5′-AACCTTGACCCCGGAGGCT-CAT-3′. First- and second-strand cDNA synthesis were carried out using RT-PCR kits (Perkin-Elmer Corp.). The cDNA was circularized at 16°C overnight using T4 DNA ligase (Life Technologies, Inc., Grand Island, NY). The circularized cDNA was subjected to PCR using primer sets RACE-s and RACE-as. These primers were complementary to PR cDNA and numbered at the 5′ base according to Kastner et al. (4).

Sequencing. For confirmation of MSP and 5′RACE, the PCR products were purified by QIAquick PCR Purification kit (Qiagen, Valencia, CA) and applied to second-PCR. Thirty ng of PCR products were used as a template for sequencing (23). Double-strand sequence analysis was performed using each

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Cycle</th>
<th>Final incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRB-Wf</td>
<td>5′-ACGGGCTACTTCTTCTTCCG-3′</td>
<td>94°C, 30 s</td>
<td>57°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Wr</td>
<td>5′-TGGAAATGCCGTCCCTACGG-3′</td>
<td>94°C, 30 s</td>
<td>41°C, 30 s</td>
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<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Uf</td>
<td>5′-ATGTTATTTTTTTTTTTTTG-3′</td>
<td>94°C, 30 s</td>
<td>45°C, 45 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Ur</td>
<td>5′-TAAATATACCTTACCCCTCA-3′</td>
<td>94°C, 30 s</td>
<td>45°C, 45 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Mf</td>
<td>5′-ACGGTTATTTTTTTTTTTG-3′</td>
<td>94°C, 30 s</td>
<td>45°C, 45 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Mr</td>
<td>5′-TAAATATACCCCTACCA-3′</td>
<td>94°C, 30 s</td>
<td>45°C, 45 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Wf</td>
<td>5′-GAGCTCTGCCGCGCAGTGACG-3′</td>
<td>94°C, 30 s</td>
<td>57°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Wr</td>
<td>5′-CCGCAAATTAGTGACACCGG-3′</td>
<td>94°C, 30 s</td>
<td>45°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
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<tr>
<td>PRB-Uf</td>
<td>5′-TGTATGGTGTTAGTATGACG-3′</td>
<td>94°C, 30 s</td>
<td>57°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
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<tr>
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<td>94°C, 30 s</td>
<td>45°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>PRB-Mf</td>
<td>5′-CGAATTATTACGACCGG-3′</td>
<td>94°C, 30 s</td>
<td>51°C, 45 s</td>
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<td>72°C, 8 min</td>
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<td>51°C, 45 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
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<td>RACE-s</td>
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<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>RACE-as</td>
<td>5′-AACGCGGCGCTGGGCTGGG-3′</td>
<td>94°C, 30 s</td>
<td>51°C, 45 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>RT-PCR AB-f</td>
<td>5′-ACGCCGCGCGCTGGGCGAGG-3′</td>
<td>94°C, 30 s</td>
<td>58°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>RT-PCR AB-r</td>
<td>5′-CCACCAAGCGCGCGAGG-3′</td>
<td>94°C, 30 s</td>
<td>58°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>RT-PCR B-f</td>
<td>5′-ACTGACTCTGAAGGGAAAGAGT-3′</td>
<td>94°C, 30 s</td>
<td>60°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>RT-PCR B-r</td>
<td>5′-GTCTGCTCTGCGCAGG-3′</td>
<td>94°C, 30 s</td>
<td>60°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>β actin-f</td>
<td>5′-AAGCCCAACCGCGCAAGAGT-3′</td>
<td>94°C, 30 s</td>
<td>52°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
<tr>
<td>β actin-r</td>
<td>5′-TGCGTGGAGCTTCTTATGAG-3′</td>
<td>94°C, 30 s</td>
<td>52°C, 30 s</td>
<td>72°C, 60 s</td>
<td>40</td>
<td>72°C, 8 min</td>
</tr>
</tbody>
</table>
PRB and PRA, respectively, according the data at 1 for CpG dinucleotide in the RACE-as. By this method, the product from PRB is longer than PRA. A scheme of modified 5'RACE method for distinguishing each mRNA expression is shown. mRNA was reverse transcribed using the oligonucleotide RACE-REV. First- and second-strand cDNA synthesis were carried out. The two different first-strand cDNAs from PRA and PRB are produced by reverse transcription using 5'RACE-REV. The cDNA was circularized using T4 DNA ligase. The circularized cDNA was subjected to PCR using primer sets RACE-s and RACE-as. By this method, the product from PRB had longer bands than PRA. A schematic frequency for CpG dinucleotide in the PR gene is also shown.

RESULTS

Cell Lines. Fig. 1 shows the schematic presentation of upstream regions of the PR genes. The positions and orientation of MSP and RT-PCR primers are indicated. Fig. 1 also shows a modified 5'RACE method. We used a modified 5'RACE method for distinguishing each mRNA expression because PRA has no unique sequences compared with PRB mRNA. mRNA was reverse-transcribed using an oligonucleotide, RACE-REV. First- and second-strand cDNA synthesis were carried out. The cDNA was circularized using T4 DNA ligase. The circularized cDNA was subjected to PCR using primer sets RACE-s and RACE-as. By this method, the product from PRB had longer bands than that of PRA.

MSP and RT-PCR were performed using these cell lines treated with and without the demethylating reagent 5-azaC (Table 2; Fig. 2). By MSP without 5-azaC treatment, only PRB was methylated in all of the cell lines, although PRA was unmethylated (Table 2; Fig. 2A). By RT-PCR, no PRB expression was found, although PRA expression was found in all UEC cell lines (Table 2; Fig. 2B). Treatment of cell lines with 5-azaC restored PRB expression in all cancer cell lines (Table 2; Fig. 2). Fig. 2C shows expression of PRA and PRB in Ishikawa cells using the modified 5'RACE. A shorter band derived from PRA was observed after 5-azaC treatment because of inactivation of PRB. However, after 5-azaC treatment, both longer and shorter bands were observed because of re-expression of PRB. We also investigated PR genes using the W primer sets, and no mutation or loss of heterozygosity was found in the results (results not shown).

MSP in Cancerous and Normal Samples. All normal samples had only unmethylated bands of PRA and PRB by MSP (Table 3). Sixty-two of 83 cancer samples (74.7%) had only methylated bands of PRB, although all normal samples (100%) had only unmethylated bands. All cancer and normal samples (100%) had only unmethylated bands of PRA. There was a significant difference in methylation status of PRB in cancerous and normal tissues (P < 0.001), although there was no difference in methylation status of PRA.

Immunohistochemistry in Cancerous and Normal Samples. Seventy-one of 83 cancer samples (85.4%) were positive for PRB, whereas 11 of 12 normal samples were positive for PRA (86.7%) (Table 4). To determine PR methylation and expression in normal endometrial cells, we studied 33 normal endometrial samples. Twenty-six of 29 normal samples (72.7%) were positive for PRA-B expression (Table 4; Fig. 3). A significant difference was found in PRB positivity in cancerous versus normal tissues (P < 0.001), although there was no such difference in PRA-B expression.
cancer samples (91.7%) that had only unmethylated alleles were positive (P < 0.001; Table 5).

**Pairs of Cancer and Normal Samples from the Same Patient.**
To determine whether de novo methylation at PRB occurred in UEC patients, we studied 32 pairs of cancer and normal samples from the same patient (Table 6). Twenty of 32 cancer samples (62.5%) had only methylated alleles, although all 32 normal samples (100%) had only unmethylated alleles (Table 6). Seven of 8 cancer samples (87.5%) that had only unmethylated PRB alleles were PRB positive.

All cancer samples that had only methylated alleles were negative for PRB expression. The loss of unmethylated allele correlated well with negativity in immunohistochemical staining for PRB (P < 0.001).

Fig. 4 shows typical results of the MSP assay of the PR gene in a pair of cancer and normal tissue from the same patient. Only unmethylated bands of the promoter A were observed in both normal (Lanes 1 and 2) and cancer tissues (Lanes 3 and 4). Only methylated alleles of PRB were observed in cancer samples (Lanes 9 and 10), although only unmethylated alleles of PRB were observed in normal tissue (Lanes 7 and 8). Fig. 5 shows direct sequencing of the PRB bands. CpG sites are underlined, and all cytosines are deaminated and converted to thymines in the unmethylated allele, whereas 5-methylcytosines remain unaltered in the methylated allele.

**DISCUSSION**

The PR is a member of a closely related subgroup of nuclear receptors that includes the androgen, mineral corticoid, and glucocorticoid receptors. Within this subgroup, PR is unique because it occurs in target tissues as two distinct subtypes, PRA and PRB, of M, 94,000 and M, 114,000, respectively (4, 5). The PRB isoform contains an NH2-terminal fragment of 164 amino acids, which is absent in the PRA isoform. Both isoforms arise by different initiation of translation from the same mRNA or by transcription from different promoters within the same gene. These promoters, which regulate the synthesis of specific transcripts of PRA and PRB, are regulated independently.
It is likely that the expression levels of PRA and PRB can differ with respect to each other in certain target tissues (7, 8). PRA and PRB also differ in their ability to inhibit the activity of ER in such a way that is both promoter and cell type specific. The functional implications of NH₂-terminal isoforms are not restricted to PR. Androgen receptor mRNA isoforms, which are different within the region coding for the NH₂-terminal portion of the protein, have been described and are either developmentally regulated or are expressed in differentiated tissues as in Xenopus laevis (6, 7).

The specific roles of these two PR subtypes are unclear. However, the relative levels of PRA and PRB in cells are critical for appropriate cellular response to progesterone (10, 11). Kumar et al. (16) revealed selective down-regulation of PRB in human UEC cells. They hypothesized that the PRA:PRB ratio may be abnormal in endometrial cancer, which may lead to a lack of normal progesterone protection against the growth-promoting effects of E₂.

In this study, we investigated the methylation status of PRA and PRB in UEC cell lines using MSP. PRB is methylated in all human UEC cells, whereas PRA is unmethylated. We also investigated the expression of two isoforms of PR in UEC cell lines and found that PRB is inactivated. The data by the 5’RACE method confirmed only that PRB is inactivated in all cell lines and distinguished PRA and PRB independently.

To understand whether PRB mRNA expression is inactivated by methylation, we treated cells with 5-azaC. The treatment of 5-azaC restored PRB expression in all PRB-negative cell lines. Our results showed clearly that there is a close relationship between the selective inactivation of the PRB gene and abnormal methylation of PRB.

The molecular mechanisms of PRB inactivation in endometrial cancer are unknown. Several investigators have shown that PRB is protective against the hyperplastic effects of E₂ (5, 6). It is well known that induction of endometrial cancer is related to hyperestrogenism (2). The tumorigenic effect of estrogen is completely reversed by the addition of progestational agents (3). Progesterone treatment is effective in decreasing the growth of endometrial tumors that express PRs. The activity of PR strongly correlates with successful endocrine treatment and patient’s survival (3). It is possible that the PRB inactivation in endometrial cancer cannot protect proliferation of endometrial cells induced by E₂ and thus cannot prevent carcinogenesis of the uterine endometrial cells.

De novo methylation in the islands of several genes in vitro can block transcription of downstream sequences (18–22). Abnormal methylation has been observed for several genes in cancer cells (25–27). Such methylation has been identified recently as an alternate

![Fig. 4. Methylation status of promoter for PRA and PRB in UEC samples. L, 25-bp ladder marker; U, unmethylated bands; M, methylated bands; N, normal tissues; C, cancerous tissues. Lanes 1 and 2 are unmethylated and methylated PRA alleles in normal samples, respectively. Lanes 3 and 4, unmethylated and methylated PRA alleles in cancer samples, respectively. For these samples, PRA promoter primers were used. Lanes 5 and 6, unmethylated and methylated PRB alleles in normal samples, respectively. Lanes 7 and 8, unmethylated and methylated PRB alleles in cancer samples, respectively. For these samples, PRB primers were used.](image)

![Fig. 5. Examples of direct DNA sequencing chromatogram for each type of PRB promoter region. a, unmethylated allele. b, methylated allele. c, unmodified allele. CpG sites are underlined, and all cytosines are deaminated and converted to thymines in the unmethylated allele, whereas 5-methylcytosines remain unaltered in the methylated allele (*).](image)
mechanism of inactivating tumor suppressor genes during the development of cancer (19, 20, 22, 27).

CpG islands are found in other members of the hormone receptor superfamily including androgen, progesterone, and estrogen receptor genes (4, 20–22). We have recently observed methylation of the estrogen receptor promoter in prostate cancer during carcinogenesis (28). In that report, we describe how selective PRB hypermethylation is associated with the loss of PRB expression in the endometrial cancer cell lines and endometrial cancer tissues (28).

MSP is a new technology for the detection of gene methylation using small amounts of DNA (29–31). This technique offers a highly sensitive approach. MSP also has the potential to define tumor suppressor genes and provides a new strategy for tumor detection research. Lapidus et al. (22) revealed that CpG islands in exon 1 of the PR gene is methylated in a significant fraction of primary human breast cancer. Thus, methylation-sensitive restriction sites in the PR gene CpG island are not methylated in normal breast specimens but methylated in PR-negative human breast tumors. Their data demonstrate that methylation of the PR gene CpG islands is associated with the lack of PR gene expression in a significant fraction of human breast cancers. However, they investigated only methylation status of exon 1 for both isoforms, because the promoter regions of PRA and PRB lack restriction enzyme recognition sites (22).

In this study, we investigated the methylation status of PRA and PRB separately using 83 cancerous and 33 normal endometrial tissues from UEC patients. 74.7% of cancer samples had only methylated PRB alleles, although all normal samples had unmethylated PRB alleles. No significant changes were observed in the methylation status of PRA in normal and cancer samples. This is the first report that the selective methylation and silencing of PRB is observed in UEC.

REFERENCES

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Hypermethylation in Human Uterine Endometrial Cancer

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