The Relationship between the Methylation of Promoter Regions of Tumor Suppressor Genes \textit{PTEN} and \textit{APC} with Endometrial Cancer

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Abstract

\textbf{Background:} Endometrial neoplasms is one of the most typical gynecologic diseases with harmful effects. Promoter hypermethylation is an important mechanism of the inactivation of tumor suppressor genes in endometrial neoplasms. Epigenetic changes of the \textit{PTEN} and \textit{APC} genes have shown to be present in various cancers. Therefore, in this study, we have investigated the association between the promoter hypermethylation of \textit{PTEN} and \textit{APC} genes with endometrial neoplasms. \textbf{Methods:} For this study, 28 patients with endometrial neoplasms as well as 22 controls were studied. Analysis of the promoter methylation regions of \textit{PTEN} and \textit{APC} genes were performed by Methylation-Specific PCR. \textbf{Results:} The frequency of \textit{PTEN} and \textit{APC} genes promoter methylation was 28.57\% and 17.86\% in tumor tissues, and 11.54\% and 3.85\% in blood samples, respectively. We found a significant relationship between blood and tissue in \textit{PTEN} methylation (p = 0.0353). Additionally, we determined a closely significant difference between normal tissue and tumor tissue of the \textit{PTEN} gene (p = 0.0787) and blood and tissue samples of the \textit{APC} gene in methylated promoter regions (p=0.0623). Furthermore, these results suggest that there is no significant relationship between the promoter methylation of \textit{PTEN} and \textit{APC} with clinical characteristics. \textbf{Conclusion:} DNA methylation deficiency is a well known highlighted factor in tumorigenesis, therefore the promoter hypermethylation of \textit{PTEN} and \textit{APC} can be indicated as a risk factor in endometrial neoplasms.

\textbf{Keywords:} Endometrial neoplasms, DNA methylation- \textit{APC}- \textit{PTEN}

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Introduction

One of the most common forms of cancers in females, which is usually recognized at early stages, is endometrial cancer (EC) (Markowska et al., 2014). Collected information has revealed that the prevalence of endometrial cancer with stage I and II was 73\% and 10\%, respectively (Trimble et al., 2005; Creasman et al., 2006). There are two variants of endometrial cancer with diversity in their clinicopathologic features. Type I tumors (endometrioid cancers) are completely differentiated and estrogen-related. On the other hand, type II tumors (non-endometrioid cancers) are non-estrogen-related (Tao and Freudenheim, 2010). Major risk factors in endometrial cancer include the estrogen element, mismatch repair disorders, microsatellite instability, and epigenetic variation (Banno et al., 2014; Mutter et al., 1996). Similar to other types of cancer, endometrial carcinoma can be caused by the aggregation of genetic mutations (Feng et al., 2012).

Genomic DNA methylation depends on the addition of methyl groups at \textit{CpG} sites by DNA methyltransferase. DNA methylation is normally visible in promoters and is important for gene expression (Muraki et al., 2009). The inactivation of tumor suppressor genes is one of the reasons of tumor formation (Herman and Baylin, 2003). Phosphatase and tensin homologue (\textit{PTEN}) is a type of tumor suppressor gene that is in 10q23.31. It is vital for the inhibition of cell migration. \textit{PTEN} is a type of lipid 3-phosphatase and can moderate different types of cell-survival pathways (Waite and Eng, 2002). The PI3K–AKT pathway is negatively regulated by the \textit{PTEN} protein. 34–55\% of endometrial cancers are reported with mutations in \textit{PTEN} (Risinger et al., 1997; Kong et al., 1997). The inactivation of \textit{PTEN} in endometrial cancer could be investigated by promoter hypermethylation, instability of the protein, and a change in the regulation of the gene (Zhang and Yu, 2010). Many surveys have reported that 20\% of type I endometrial cancers are methylated in the promoter region of the \textit{PTEN} and \textit{APC} genes (Macdonald et al., 2004; Salvesen et al., 2001). \textit{PTEN} is a second messenger of phosphatidylinositol 3-kinase (PI3K) that negatively regulates serine/threonine kinase Akt. The phosphorylation of Akt modifies the
activities of many downstream proteins that regulate the growth of cells and inhibit apoptosis (Oda et al., 2005; Velasco et al., 2006; Dubrovska et al., 2009; Rychahou et al., 2008).

The adenomatous polyposis coli (APC) is a tumor suppressor gene that is located in 5q21-22 (Aoki and Takeo, 2007). Alterations of the APC gene has been reported in 80% of colon cancers (Powell et al., 1992; Nagase and Nakamura, 1993). The Wnt pathway is regulated by mutations in the APC and β-catenin genes that are also correlated with endometrial cancer (Schlosshauer et al., 2000; Kobayashi et al., 1999). Hypermethylation of the APC promoter has been reported in 20–45% of endometrial cancers (Banno et al., 2006; Yang et al., 2006). APC is shown to moderate β-catenin levels, whereas, inhibition of APC expression will lead to an increase in the levels of β-catenin, which induces the Wnt signaling pathway and eventually increases the transcriptional activity (Fearnhead et al., 2001; Behrens et al., 1996). The purpose of the present study is to evaluate the association between PTEN and APC promoter hypermethylation with endometrial cancer in the blood and tissue samples in endometrial cancer patients. Also, we aim to investigate the correlation between endometrial cancer and distinct clinical characteristics.

Materials and Methods

Research population

Twenty eight cancerous tissues along with associated clinopathological parameters and twenty six blood samples of the same individual with endometrial cancer were collected from patients who had been referred to the Mahdiyeh Hospital (Tehran, Iran), Firuzgar Hospital (Tehran, Iran), Kowsar Hospital (Ghazvin, Iran), and Pasteur Hospital (Ghazvin, Iran), between 2014 and 2016. In addition, twenty-two normal tissues were sampled as the control group. Informed consent was signed by the patients. The average age was 65.5 years for both groups (range, 38–76 years). None of the patients had received chemotherapy. They were examined in the Biology Research Center of Islamic Azad University, Zanjan (Iran). The clinical diagnosis of endometrial cancer was in accordance with the criteria of the International Federation of Gynecology and Obstetrics (FIGO). The clinicopathologic traits were diagnosed by expert gynecologists.

DNA isolation and methylation-specific PCR

Tissue samples were cut into 10 μm segments and used for DNA isolation (Cat.NO.180134, QIAGEN Inc, Valencia, CA) and also genomic DNA was extracted from EDTA-blood samples using the cinnaClon Genomic DNA purification Kit (Cat.No.PR881612) following the manufacturer’s instructions. Sodium bisulfite converts unmethylated cytosine to uracil. The effects of bisulfite manufacturer’s instructions. Sodium bisulfite converts DNA purification Kit (Cat.No.PR881612) following the and also genomic DNA was extracted for DNA isolation (Cat.NO.180134, QIAGEN Inc, Valencia, CA) and also genomic DNA was extracted from EDTA-blood samples using the cinnaClon Genomic DNA purification Kit (Cat.No.PR881612) following the manufacturer’s instructions. Sodium bisulfite converts unmethylated cytosine to uracil. The effects of bisulfite conversion of cytosine to uracil (Gravina et al., 2016). Afterwards, the buffer BL/carrier RNA solution was used to increase extracted DNA levels (Cat. No.59104, QIAGEN Inc, Valencia, CA) (Gravina et al., 2015). Forward and reverse primers were designed for unmethylated and methylated promoter regions by Gene runner and meth primer software after obtaining gene sequences from Gene Bank (http://www.ncbi.nlm.nih.gov). The primer sequences are shown in Table 1. Amplification was carried by gradient PCR, using a model of PCR gradient thermo cycler (Eppendorf, Germany). MSP amplification was performed in a final volume of 20 μL containing 1X PCR Master Mix (CinnaGen PCR Master Mix, Iran), 50 ng of template bisulfite converted DNA, and 10 pmol of forward and reverse primers each (Gen Fanavar, Iran). MSP amplifications was performed as follows: the first denaturation cycle of DNA at 95°C for 5 min followed by 34 cycles, each consisting of 45 s denaturation at 95°C, 45 s annealing at 59°C for PTEN (M), 57°C for PTEN (U), 54°C for APC (M), 57°C for APC (U), and 45 s extension at 72°C, with the final extension cycle of 72°C for 5 min. The PCR products were analyzed by the electrophoresis of agarose gel (2.5% agarose), stained with DNA safe stain and visualized by a UV transilluminator. For MSP confirmation and correct determination of methylated, hemimethylated, and non methylated genotypes, modified genomic DNA samples from regulatory CpG islands of both normal endometrium and cancerous tissue were analyzed by sequencing for both PTEN and APC genes.

Statistical analysis

The statistical comparison was calculated by Pearson’s chi-square. The data analysis was performed by SPSS20. P-values <0.05 were statistically significant. The rates of odds ratio and 95% confidence intervals were assayed by logistic regression.

Results

Determination of clinicopathological features

The samples consisted of 25 endometrioid carcinomas [endometrial adenocarcinoma:19 FIGO grade 1 (stage IA, IB), 4 FIGO grade 2, 2 FIGO grade 3 (IIIB)], and 3 non-endometrioid carcinomas [serous papillary:3 FIGO grade 3 (stage IIIA)]. Nine of twenty-five (36%) endometrioid carcinomas [serous papillary:3 FIGO grade 3 (stage IIIA)]. Nine of twenty-five (36%) endometrioid carcinomas showed metastasis. In addition, non-endometrioid carcinomas lacked signs of any metastasis.

Analysis of promoter methylation in PTEN and APC genes

Methylation specific PCR was used to assay the role of the methylation status of PTEN and APC promoters in endometrial cancer. The frequencies of PTEN and APC methylation in promoter regions have been shown in Table 2. Hypermethylation of PTEN was observed in 28.57% of tumor tissues and 4.54% of normal tissues (Figure 1, 3). The results were closely linked to being statistically significant between tumor tissues and normal tissues (OR=2.4377, 95%CI [1.0635-5.5876], p = 0.0353) (Table 2). There was a significant increase of PTEN methylation in patients’ blood (11.54%) compared to tumor tissues (28.57%). There was a significant difference in the methylation of the PTEN promoter between the patients’ blood and tumor tissues (OR=2.4377, 95% CI [1.0635-5.5876], p = 0.0353) (Table 2). Promoter methylation
The Relationship between the Methylation of Promoter Regions of Tumor Suppressor Genes PTEN and APC

The methylation of the PTEN gene was shown in 17.86% of tumor tissues and 4.55% of normal tissues (Figure 2, 3). The study showed that APC methylation was not significantly correlated between tumor tissues and normal tissues (OR=0.7073, 95% CI [0.2995-1.6703], p = 0.4296) (Table 2). Among the patients’ blood, the frequency of promoter methylation in the APC gene was 3.85% (Figure 2, 3). Results indicate that the promoter methylation analysis of the APC gene between tumor tissues and patients’ blood is statistically significant (OR=0.4047, 95% CI [0.1563-1.0476], p=0.0623) (Table 2). The association between the methylation of PTEN and APC promoters with clinicopathological features was analyzed in the endometrial cancer. We found no significant correlations between clinicopathological features, including age, tumor grade, tumor stage, histologic type, depth of myometrial invasion, stage, and grade. The methylation-specific PCR analysis of the PTEN and APC promoters was performed using Primer pairs as shown in Table 1.

Table 1. Primer Pairs Used for Methylation-Specific PCR

| Primer                  | Primer sequence (5'-3')                  | Size (bp) |
|-------------------------|------------------------------------------|-----------|
| PTEN promoter methylated (M) | F:GGT TTC GGA GTG CGT CGGC               | 19        |
|                         | R:CAA CCG AAT ATT AAC TAC TAC GAC        | 24        |
| PTEN promoter unmethylated (U) | F:TGG GTT TTG GAG GTT GTT GTT           | 21        |
|                         | R:ACT TAA CTC TAA ACC ACA ACC            | 21        |
| APC promoter methylated (M) | F:TAT TGC GGA GTT CGG GTC               | 18        |
|                         | R:TCG ACG AAC TCC CGA CGA               | 18        |
| APC promoter unmethylated (U) | F:GTG TTT TAT TGT GGA GTG TGG GTT      | 24        |
|                         | R:CCA ATC AAC AAA CTC CCA ACA A         | 22        |

Figure 1. Methylation-Specific PCR Analysis of PTEN Promoter: A1: using unmethylated promoter primers; Lane M: 50bp ladder; Lane 1: absence of PCR products: methylated promoter; Lanes 2, 3, 4: unmethylated PCR products (86bp). B1: using methylated promoter primers; Lane M: 50bp ladder; Lanes 1, 2, 4: methylated PCR products (105bp); Lane 3: absence of PCR products: unmethylated PCR products.

Figure 2. Methylation-Specific PCR Analysis of APC Promoter: A2: using unmethylated promoter primers; Lane M: 50bp ladder; Lanes 1, 2, 3, 4: unmethylated PCR products (89bp). B2: using methylated promoter primers; Lane M: 50bp ladder; Lanes 1, 2: absence of PCR products: unmethylated PCR products; Lane 3: methylated PCR products (93bp).

Table 2. The Methylation Status of the Promoter Region of PTEN and APC Genes in the Study Population

| Gene | Study population (number) | Methylated number (%) | Hemi-methylated number (%) | Non-methylated number (%) | OR       | 95%CI       | P-value |
|------|---------------------------|-----------------------|----------------------------|---------------------------|----------|-------------|---------|
| PTEN | Normal tissues (22)       | 1 (4.54%)             | 13 (59.10%)                | 8 (36.36%)                | 2.0765   | 0.9197-4.6887 | 0.0787  |
|      | Tumor tissues (28)        | 8 (28.57%)            | 13 (46.43%)                | 7 (25%)                   | 2.0765   | 0.9197-4.6887 | 0.0787  |
|      | Patients’ blood (26)      | 3 (11.54%)            | 23 (88.46%)                | 0 (0%)                    | 2.4377   | 1.0635-5.5876 | 0.0353  |
| APC  | Normal tissues (22)       | 1 (4.55%)             | 4 (18.18%)                 | 17 (77.27%)               | 0.4047   | 0.1563-1.0476 | 0.0623  |
|      | Tumor tissues (28)        | 5 (17.86%)            | 5 (17.86%)                 | 18 (64.28%)               | 0.7073   | 0.2995-1.6703 | 0.4296  |
|      | Patients’ blood (26)      | 1 (3.85%)             | 7 (26.92%)                 | 1 (69.23%)                | 0.4047   | 0.1563-1.0476 | 0.0623  |
invasion, and metastasis in endometrial cancer (P>0.05) (Table 3). In addition, the correlation between promoter hypermethylation of the PTEN and APC genes with clinical characteristics, including diabetes, high weight, high blood pressure, and menstrual disorder was evaluated. Results show there was no significant difference between clinical characteristics and promoter hypermethylation of the PTEN and APC genes in endometrial cancer (P>0.05) (Table 4).

**Discussion**

Aberrant DNA hypermethylation of tumor-suppressor genes is a prevalent molecular change in the early stages of cancers and it can be highlighted as a biomarker in the detection and treatment of tumors (laird., 2003). Epigenetic deficiencies with promoter hypermethylation have an important role in the inactivation of tumor suppressor genes in cancer (Salvesen et al., 2001). This study investigated the methylation of the promoter regions of the PTEN and APC genes in endometrial cancer. Considering circulating tumor cells are present

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**Table 3. Relationship between PTEN and APC Promoter Methylation and Clinicopathological Features in Endometrial Carcinoma Patients**

| Parameters                        | Blood samples | Tissue samples |
|-----------------------------------|---------------|---------------|
|                                   | PTEN          | APC           | PTEN          | APC           |
| Total (tissue:n= 28, Blood: n=26) | Number of samples (%) | Number of samples (%) |
| Age (yr)                          |               |               |
| <50 (4)                           | 2 (40%)       | 1 (25%)       | 1 (25%)       | 2 (50%)       |
| >50 (24)                          | 12 (57.1)     | 3 (14.3%)     | 13 (54.2)     | 6 (25%)       |
| p=0.49                            |               | p=0.75        | p=0.28        | p=0.31        |
| Tumor grade                       |               |               |
| G1 (19)                           | 9 (52.9%)     | 3 (17.6%)     | 9 (50%)       | 3 (16.7%)     |
| G2 (4)                            | 3 (75%)       | 1 (25%)       | 3 (60%)       | 2 (40%)       |
| G3 (5)                            | 3 (60%)       | 1 (20%)       | 3 (60%)       | 1 (25%)       |
| p=0.72                            |               | p=0.95        | p=0.88        | p=0.53        |
| Tumor stage                       |               |               |
| IA (17)                           | 9 (56.3%)     | 4 (25%)       | 9 (52.9%)     | 3 (17.6%)     |
| IB (2)                            | 1 (50%)       | 0 (0%)        | 0 (0%)        | 0 (0%)        |
| II (4)                            | 2 (66.7%)     | 1 (33.3%)     | 2 (50%)       | 1 (25%)       |
| IIIA (3)                          | 2 (66.7%)     | 1 (33.3%)     | 2 (67%)       | 2 (67%)       |
| IIIB (2)                          | 2 (100%)      | 0 (0%)        | 2 (100%)      | 0 (0%)        |
| p=0.81                            |               | p=0.81        | p=0.37        | p=0.30        |
| Histologic type                   |               |               |
| Endometrioid type (25)            | 13 (56.5%)    | 4 (17.4%)     | 13 (52%)      | 5 (20%)       |
| Nonendometrioid type (3)          | 2 (66.7%)     | 1 (33.3%)     | 2 (66.7%)     | 1 (33.3%)     |
| p=0.74                            |               | p=0.51        | p=0.63        | p=0.60        |
| Depth of myometrial invasion      |               |               |
| Negative (3)                      | 2 (66.7%)     | 0 (0%)        | 2 (66.7%)     | 2 (66.7%)     |
| <50 % (15)                        | 8 (57.1%)     | 3 (21.4%)     | 7 (46.7%)     | 2 (13.3%)     |
| >50 % (10)                        | 6 (66.7%)     | 2 (22.2%)     | 5 (50%)       | 3 (30%)       |
| p=0.88                            |               | p=0.67        | p=0.82        | p=0.14        |
| Metastase                         |               |               |
| Negative (19)                     | 10 (55.6%)    | 4 (22.2%)     | 11 (57.9%)    | 4 (21.1%)     |
| Positive (9)                      | 5 (62.5%)     | 1 (12.5%)     | 4 (44.4%)     | 2 (22.2%)     |
| p=0.74                            |               | p=0.56        | p=0.50        | p=0.94        |

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**Figure 3. Methylation-Specific PCR Analysis of APC and PTEN Promoters: APC was unmethylated, PTEN was hemi-methylated; M, 50bp ladder ; u, reactions in unmethylated promoter primers; m, reactions in methylated promoter primers.**
Table 4. Association between Clinical Characteristics of the Study Groups with Endometrial Cancer

| Parameters           | Blood samples | Tissue samples |
|----------------------|---------------|----------------|
|                      | PTEN          | APC            | PTEN            | APC            |
| Diabetes             |               |                |                |                |
| Negative (17)        | 8 (47%)       | 4 (23.5%)      | 9 (52.9%)       | 4 (23.5%)      |
| Positive (11)        | 7 (63.6%)     | 2 (18.2%)      | 6 (54.5%)       | 2 (18.2%)      |
| p=0.390             | P=0.736       | P=0.934        | p=0.736         |
| High weight          |               |                |                |                |
| Negative (9)         | 5 (55.6%)     | 2 (22.2%)      | 4 (44.4%)       | 2 (22.2%)      |
| Positive (19)        | 10 (52.6%)    | 3 (15.8%)      | 10 (52.6%)      | 9 (47.4%)      |
| p=0.885             | p=0.678       | p=0.686        | p=0.203         |
| High blood pressure  |               |                |                |                |
| Negative (20)        | 9 (45%)       | 3 (15%)        | 8 (40%)         | 4 (20%)        |
| Positive (8)         | 6 (75%)       | 2 (25%)        | 6 (75%)         | 2 (25%)        |
| p=0.150             | p=0.533       | p=0.094        | p=0.771         |
| Menstrual disorder   |               |                |                |                |
| Negative (25)        | 20 (80%)      | 6 (24%)        | 15 (60%)        | 6 (24%)        |
| Positive (3)         | 3 (100%)      | 2 (66.7%)      | 2 (66.7%)       | 1 (333%)       |
| p=0.393             | p=0.122       | p=0.823        | p=0.724         |

In our estimate, the prevalence of PTEN methylation was 11.54% and 28.57% in patients’ blood and tumor tissues, respectively. QI et al., (2014) showed that the frequency of PTEN promoter hypermethylation was 62% in tumor tissues of patients with cervical cancer where the methylation of the PTEN promoter was significantly related to the tumor grade, metastasis, and tumor stage (P<0.05). They found a significant association between the promoter methylation of PTEN and cervical cancer (P=0.042). They also suggested that promoter hypermethylation could be a key mechanism of PTEN inactivation in cervical cancer. We found a meaningful association between the hypermethylation of the PTEN promoter and endometrial cancer in patients’ blood. The APC/β-catenin pathways were initially reported in the field of endometrial cancer (Aoki and Taketo, 2007; Moreno-Bueno et al., 2002). Guo et al., (2014) reported a significant association between the APC promoter methylation and non-small cell lung cancer (NSCLC) (OR=3.79, 95% CI [2.22 - 6.45], P < 0.0001). According to Ignatov (2010), 56.9% of endometrial carcinomas show APC promoter methylation in tumor tissues. They revealed that there was no relationship between histological type, histological grading, and methylation of the APC gene. However, there was a significant reverse relationship between metastasis and APC methylation (p=0.002). In our study, promoter methylation in the APC gene was found in 3.85% and 17.86% of patients’ blood and tumor tissues, respectively. Richiardi (2009), reported that APC hypermethylation had an important role in prostate cancer and there was a significant association between prostate cancer and APC promoter hypermethylation (OR=1.49, 95% CI [1.11 to 2.00], P = 0.047). Our results closely linked a statistically significant difference in the promoter methylation analysis of the APC gene between tumor tissues and patients’ blood. Moreover, we found
no significant relationship between clinicopathological features and \(\text{APC}\) methylation (\(P=0.05\)). Based on our study, promoter hypermethylation of \(\text{APC}\) and \(\text{PTEN}\) genes may be a biomarker in the diagnosis of endometrial cancer. More studies with larger populations and a suitable election of the case-control study will be essential to understand the role of hypermethylation of the \(\text{APC}\) and \(\text{PTEN}\) genes as biomarkers in endometrial cancer.

In conclusion, promoter methylation of tumor-suppressor genes is a typical epigenetic change in endometrial cancer. Hypermethylation of \(\text{PTEN}\) and \(\text{APC}\) in the promoter region may have a significant effect in the development of endometrial cancer. In our study, there was a significant relationship between blood and tissue in \(\text{PTEN}\) methylation (OR=2.4377, 95% CI [1.0635-5.5876], \(p = 0.0353\)). However, there was no evidence to support an association of promoter methylation of the \(\text{APC}\) gene in tumor tissues with endometrial cancer (OR=0.7073, 95% CI [0.2995-1.6703], \(p = 0.4296\)). Furthermore, there was a closely significant difference between the methylated regions in blood and tissue samples of the \(\text{APC}\) gene as well as normal tissue and tumor tissue of the \(\text{PTEN}\) gene (OR=0.4047, 95% CI [0.1563-1.0476], \(p = 0.0623\)); (OR=2.0765, 95% CI [0.9197-4.6887], \(p = 0.0787\)), respectively.

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Conflict of Interest

The authors declare no conflict of interest.

References

Aoki K, Takeko MM (2007). Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. J Cell Sci, 120, 3327-35.

Banno K, Yanokura M, Iida M, Masuda K, Aoki D (2014). Carcinogenic mechanisms of endometrial cancer: involvement of genetics and epigenetics. J Obstet Gynaecol Res, 40, 1957-67.

Banno K, Yanokura M, Susumu N, et al (2006). Relationship of the aberrant DNA hypermethylation of cancer-related genes with carcinogenesis of endometrial cancer. Oncol Rep, 16, 1189-96.

Behrens J, von Kries JP, Köhl M, et al (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. Nature, 382, 638-42.

Cresman WT, Odicino F, Maisonneuve P, et al (2006). Carcinoma of the corpus uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet, 95, 105-43.

Dubrovskas A, Kim S, Salamone RJ, et al (2009). The role of \(\text{PTEN/Akt/PI3K}\) signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proc Natl Acad Sci U S A, 106, 268-73.

Fearnhead NS, Britton MP, Bodmer WF (2001). The ABC of \(\text{APC}\). Hum Mol Genet, 10, 721-33.

Feng ZZ, Chen JW, Yang ZR, Lu GZ, Cai ZG (2012). Expression of \(\text{PTTG}\) and \(\text{PTEN}\) in endometrial carcinoma: correlation with tumorigenesis and progression. Med Oncol, 29, 304-10.

Gao Q, Ye F, Xia X, et al (2009). Correlation between \(\text{PTEN}\) expression and \(\text{PI3K/Akt}\) signal pathway in endometrial carcinoma. J Huazhong Univ Sci Technol Med Sci, 29, 59-63.

Gravina S, Ganapathi S, Vijg J (2015). Single-cell, locus-specific bisulfite sequencing (SLBS) for direct detection of epimutations in DNA methylation patterns. Nucleic Acids Res, 43, 93.

Guo S, Tan L, Pu W, et al (2014). Quantitative assessment of the diagnostic role of \(\text{APC}\) promoter methylation in non-small cell lung cancer. Clin Epigenetics, 6, 5.

Herman JG, Baylin SB (2003). Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med, 349, 2042-54.

Ignatov A, Bischoff J, Ignatov T, et al (2010). \(\text{APC}\) promoter hypermethylation is an early event in endometrial tumorigenesis. Cancer Sci, 101, 321-7.

Kobayashi K, Sagae S, Nishioya Y, Tokino T, Kudo R (1999). Mutations of the beta-catenin gene in endometrial carcinomas. Jpn J Cancer Res, 90, 55-9.

Kong D, Suzuki A, Zou TT, et al (1997). \(\text{PTEN}\) is frequently mutated in primary endometrial carcinomas. Nat Genet, 17, 143-4.

Laird PW (2003). The power and the promise of DNA methylation markers. Nat Rev Cancer, 3, 253-66.

Macdonald ND, Salvesen HB, Ryan A, et al (2004). Molecular differences between \(\text{RER+}\) and \(\text{RER-}\) sporadic endometrial carcinomas in a large population-based series. Int J Gynecol Cancer, 14, 957-65.

Markowska A, Pawalowska M, Lubin J, Markowska J (2014). Signalling pathways in endometrial cancer. Contemp Oncol (Poln), 18, 143-8.

Moreno-Bueno G, Hardisson D, Sánchez C, et al (2002). Abnormalities of the \(\text{APC/beta-catenin}\) pathway in endometrial cancer. Oncogene, 21, 7981-90.

Muraki Y, Banno K, Yanokura M, et al (2009). Epigenetic DNA hypermethylation: clinical applications in endometrial cancer (Review). Oncol Rep, 22, 967-72.

Mutter GL, Boynton KA, Faquin WC, Ruiz RE, Jovanovic AS (1996). Alleloype mapping of unstable microsatellites establishes direct lineage continuity between endometrial precancers and cancer. Cancer Res, 56, 4483-9.

Nagase H, Nakamura Y (1993). Mutations of the \(\text{APC}\) gene in colorectal tumors. Cancer Res, 53, 4387-9.

Nagrath S, Sequist LV, Maheswaran S, et al (2007). Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature, 450, 1235-9.

Oda K, Stokoe D, Taketani Y, McCormick F (2005). High frequency of coexistent mutations of \(\text{PIK3CA}\) and \(\text{PTEN}\) genes in endometrial carcinoma. Cancer Res, 65, 10669-73.

Powell SM, Ziln N, Beazer-Barclay Y, et al (1992). \(\text{APC}\) mutations occur early during colorectal tumorigenesis. Nature, 359, 235-7.

Qi Q, Ling Y, Zhu M, et al (2014). Promoter region methylation and loss of protein expression of \(\text{PTEN}\) and significance in cervical cancer. Biomed Rep, 2, 653-8.

Richiardi L, Fiano V, Vizzini L, et al (2009). Promoter methylation in \(\text{APC}\), \(\text{RUNX3}\), and \(\text{GSTP1}\) and mortality in prostate cancer patients. J Clin Oncol, 27, 3161-8.

Risinger JJ, Hayes AK, Berchuck A, Barrett JC (1997). \(\text{PTEN/MMAC1}\) mutations in endometrial cancers. Cancer Res, 57, 4736-8.

Rychahou PG, Kang J, Gullati P, et al (2008). Akt2 overexpression
plays a critical role in the establishment of colorectal cancer metastasis. Proc Natl Acad Sci U S A, 105, 20315-20.
Salvesen HB, MacDonald N, Ryan A, et al (2001). PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. Int J Cancer, 91, 22-6.
Schlosshauer PW, Pirog EC, Levine RL, Ellenson LH (2000). Mutational analysis of the CTNNB1 and APC genes in uterine endometrioid carcinoma. Mod Pathol, 13, 1066-71.
Sharma G, Mirza S, Prasad CP, et al (2007). Promoter hypermethylation of p16INK4A, p14ARF, CyclinD2 and Sirt2 in serum and tumor DNA from breast cancer patients. Life Sci, 80, 1873-81.
Tao MH, Freudenheim JL (2010). DNA methylation in endometrial cancer. Epigenetics, 5, 491-8.
Trimble EL, Harlan LC, Clegg LX, Stevens JL (2005). Pre-operative imaging, surgery and adjuvant therapy for women diagnosed with cancer of the corpus uteri in community practice in the United States. Gynecol Oncol, 96, 741-8.
Velasco A, Bussaglia E, Pallares J, et al (2006). PIK3CA gene mutations in endometrial carcinoma: correlation with PTEN and K-RAS alterations. Hum Pathol, 37, 1465-72.
Waite KA, Eng C (2002). Protean PTEN: form and function. Am J Hum Genet, 70, 829-4.
Yang HJ, Liu VW, Wang Y, Tsang PC, Ngan HY (2006). Differential DNA methylation profiles in gynecological cancers and correlation with clinico-pathological data. BMC Cancer, 6, 212.
Yari M, Movafagh A, Sayad A, et al (2016). Direct bisulfite sequencing and methylation specific PCR to detect methylation of p15INK4b and F7 genes in coronary artery disease patients. J Sci I R Iran, 27, 23-9.
Zhang S, Yu D (2010). PI(3)king apart PTEN's role in cancer. Clin Cancer Res, 16, 4325-30.
Zuberi M, Mir R, Dholariya S, et al (2014). RASSF1 and PTEN promoter hypermethylation influences the outcome in epithelial ovarian cancer. Clin Ovarian Gynecol Cancer, 7, 33-9.