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# Recent findings on epigenetic gene abnormalities involved in uterine cancer (Review)

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**Abstract.** Selective aberrant genetic effects that do not depend on abnormal DNA sequences are referred to as epigenetic abnormalities and are involved in carcinogenesis. In uterine cancer, various genes involved in apoptosis, cell cycle, DNA repair, cell proliferation and cell adhesion are abnormally methylated, resulting in gene silencing. Reversal of such epigenetic abnormalities in cancer cells is a potential strategy for cancer therapy, and studies on epigenetic abnormalities and treatment methods in uterine cancer are in progress. These include the evaluation of 5-hydroxymethylcytosine, which is present in cancer tissues at lower levels compared with those in normal tissues, as a prognostic marker in cervical cancer; combination therapy with 5-azacytidine and cisplatin; combination treatment focusing on tumor necrosis factor-related apoptosis-inducing ligand in cervical cancer; studies focusing on DNA mismatch repair in endometrial cancer; and use of a demethylating agent to reactivate tumor suppressor genes and inhibit tumor proliferation. Detection of epigenetic changes using biomarkers may be used for histological classification, evaluation of disease progression and identification of compounds that are able to modulate epigenetic changes and may be useful for uterine cancer treatment.

**Contents**



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## 1. Introduction

Cervical and endometrial cancer rank fourth and fifth, respectively, in cancer prevalence by organ among women worldwide (1). Studies of oncogenic pathways have shown early and frequent DNA methylation, with the extent of this change considered to be related to severity. In both cervical and endometrial cancer, specific genes involved in various pathways are known to be methylated. In cervical cancer, these genes include tumor protein p73, fragile histidine triad (*FHIT*), death-associated protein kinase 1 and PRDI-BF and RIZ domain containing 14 in the apoptotic pathway; cyclin A1 (*CCNA1*) and double C2-like domain β in the cell cycle; adenomatous polyposis coli (*APC*) and secreted frizzled-related protein (*SFRP*) in the Wnt/β-catenin pathway; Fanconi anemia, complementation group F, O-6-methylguanine-DNA methyltransferase, human MutL homolog 1 (*hMLH1*) and *CCNA1* in DNA repair; *FHIT,* retinoic acid receptor-β and myelin and lymphocyte in the cell growth pathway; and CXC chemokine receptor 4 and cell adhesion molecule 1, which are involved in cell adhesion (2-21). In endometrial cancer, the following genes are methylated: *APC,* caspase-8, checkpoint with forkhead-associated and ring finger, *E-cadherin*, *hMLH1*, *p73,* progesterone receptor, phosphatase and tensin homologue deleted on chromosome 10, Ras association domain family 1 isoform A and thrombospondin 2 (22-47). The roles of these methylated genes are listed in Table I for cervical cancer and in Table II for endometrial cancer. Gene silencing caused by methylation may promote cancer progression, and studies on the clinical significance of gene silencing are ongoing.

## 2. Diagnosis of cervical cancer and epigenetic abnormalities

A recent study demonstrated that combined detection of methylated chromosome 3 open reading frame 18, junctional adhesion molecule 3 (*JAM3*) and ankyrin repeat domain 18C (*ANKRD18CP*) provided good diagnostic outcomes (48). The sensitivity for lesions of CIN2 or higher was 74% in this screening, which is comparable with the 79% sensitivity of the high-risk human papillomavirus (HPV) DNA test. The specificity of this screening was 76%, exceeding the value of 42% for the high-risk HPV DNA test ($P<0.05$). Combined detection using *JAM3,* glial cell line-derived neurotrophic factor

family receptor α 1 and *ANKRD18CP* has 73% sensitivity and 77% specificity, and that using *JAM3/ANKRD18CP* has 72% sensitivity and 79% specificity, which are also considered as relatively good diagnostic outcomes. Higher specificity is preferable for the prevention of unnecessary follow-up. Furthermore, if HPV vaccination is promoted, the incidence of HPV-negative cervical cancer may increase and the HPV test may become less significant. Therefore, an alternative screening method with a higher specificity for HPV is needed and the methylated markers found in the abovementioned studies are candidates for this screening.

5-Hydroxymethylcytosine (*5hmC*) has been shown to be a prognostic marker for squamous cell carcinoma of the cervix (49). *5hmC* is a cytosine modification found in various biological species that is present at high levels in human and murine nerve tissues. *5hmC* is a novel DNA modification marker in mammalian genomes and is involved in DNA demethylation in epigenetic regulation. Tet protein hydroxylates 5-methylcytosine (*5mC*) to form *5hmC*, and this oxidation pathway is involved in the activation or reduction of gene expression (50). The expression levels of *5hmC*, *5mC* and *TET1, TET2 and TET3* were determined in squamous cell carcinoma tissues from the uterine cervix in 140 patients and in normal uterine cervix tissues in 40 healthy volunteers. In addition, the prognostic values of *5hmC, 5mC and TET2* were assessed for therapeutic outcomes in squamous cell carcinoma of the cervix. The results demonstrated that *5hmC* is significantly decreased and *5mC* is significantly increased in squamous cell carcinoma of the cervix compared with normal cervical tissues. Furthermore, the expression of *TET2*, but not that of *TET1 or TET3*, was decreased in squamous cell carcinoma of the cervix. A reduced level of *5hmC* was associated with a poor outcome for patients with squamous cell carcinoma of the cervix. *5hmC* expression was an independent prognostic factor for event-free and overall survival in these patients, and is likely to be useful as a prognostic marker for cervical cancer in clinical practice.

## 3. Targeting of epigenetic abnormalities in cervical cancer therapy

A recent study demonstrated the efficacy of cisplatin combined with 5-azacytidine, which acts as a demethylating agent (51). Chemotherapy with cisplatin inhibited cervical cancer cells, but the same dose of cisplatin with added 5-azacytidine exerted a more potent inhibitory effect. Combination of another demethylating agent, 5-aza-2'-deoxycytidine, with taxol or cisplatin, also inhibited growth of drug-resistant cervical cancer cells (52), with taxol exerting a particularly strong effect. The standard of care for cervical cancer is a combination of paclitaxel and cisplatin. Therefore, potentiating the effects of these drugs and eliminating chemotherapy resistance are important clinical advances.

Combination therapy including tumor necrosis factor-related apoptosis-inducing ligand *(TRAIL)* is also effective (53). *TRAIL* is a cytokine in the tumor necrosis factor (TNF) family that induces apoptosis via its receptor in various human tumor cells, but not in normal cells. *TNFRSF10C (DcR1)* and *TNFRSF10D (DcR2)* are decoy receptors and multi-drug resistance (MDR) genes, and have been identified as epigenetically inactivated genes. *TNFRSF10C* methylation is found in precancerous lesions, which suggests that this is an early event in the cervical neoplastic process. Cervical cancer cells containing inactivated *DcR1* and *DcR2* exhibited increased *TRAIL*-induced apoptosis through activation of

Table I. Methylated genes in cervical cancer.

| Gene name | Function | (Refs.) |
|---|---|---|
| *TP73* | Apoptosis | (4) |
| *FHIT* | Apoptosis | (3,5,7,15,16) |
| *DAPK1* | Apoptosis | (3) |
| *PRDM14* | Apoptosis | (6) |
| *CCNA1* | Cell cycle, DNA repair | (8,9) |
| *DOC2B* | Cell cycle | (10) |
| *APC* | Wnt/β catenin pathway | (11) |
| *SFRP* | Wnt/β catenin pathway | (12) |
| *FANCF* | DNA repair | (13) |
| *MGMT* | DNA repair | (3,14,15) |
| *hMLH1* | DNA repair | (15) |
| *FHIT* | Cell growth | (3,5,7,15,16) |
| *RAR-β* | Cell growth | (15,17) |
| *MAL* | Cell growth | (17) |
| *CXCR4* | Cell-cell adhesion | (19) |
| *CADM1* | Cell-cell adhesion | (20) |

Table II. Methylated genes in endometrial cancer.

| Gene name | Function | (Refs.) |
|---|---|---|
| *hMLH1* | DNA repair | (22-26) |
| *MGMT* | DNA repair | (27) |
| *APC* | Wnt/β catenin pathway | (25,28) |
| *CDH* | Wnt/β catenin pathway | (29,30) |
| *E-cadherin* | Wnt/β catenin pathway | (25,29-32) |
| *PTEN* | Akt pathway | (33) |
| *RASSF1A* | Apoptosis | (30,34) |
| *CASP8* | Apoptosis | (35) |
| *GSTP1* | Apoptosis | (30,36) |
| *P73* | Apoptosis | (33) |
| *PAR-4* | Apoptosis | (37) |
| *SOX2* | Apoptosis | (38) |
| *CHFR* | Cell cycle checkpoint | (39) |
| *P14* | Cell cycle checkpoint | (40) |
| *P16* | Cell cycle checkpoint | (40,41) |
| *AR* | Receptor | (42) |
| *PR* | Receptor | (43) |
| *RARβ* | Receptor | (44) |
| *TESTIN* | Epithelial-to-mesenchymal transition | (45) |
| *GATA4* | Transcription factor | (46) |
| *TIMP3* | Proteasome system | (47) |

extrinsic apoptosis pathways (53). Therefore, patients without expression of decoy receptors are predicted to exhibit an enhanced response to combined therapy with *TRAIL*. This suggests that a strategy aimed at downregulating decoy receptors may be a feasible approach to treatment using *TRAIL*.

## 4. Diagnosis of endometrial cancer and epigenetic abnormalities

Endometrial cancer induced by epigenetic abnormalities is frequently caused by breakdown of the DNA mismatch repair (MMR) system. Under normal conditions, this system perceives DNA insertions and deletions, induces expression of repair enzymes, deletes affected DNA regions and resynthesizes damaged genes via DNA polymerases (54). If the MMR system is dysfunctional, DNA replication mistakes are not repaired and microsatellites (repeats of 1-5 bases) are likely to increase or decrease. This condition is referred to as microsatellite instability (MSI). MMR genes may be abnormally methylated and cancer develops when MSI occurs in regions of tumor suppressor genes. Numerous microsatellites are detectable by PCR and are found in 20-40% of cases of endometrial cancer (22,55-57).

Genes involved in the MMR system include *hMLH1,* MutS protein homolog 2 (*hMSH2*) and *hMSH6,* and postmeiotic segregation increased 2 (*hPMS2*) is frequently abnormally methylated (56). *hMLH1* methylation has been found in 40.1% of cases of endometrial cancer and in 14.3% of cases of atypical endometrial hyperplasia, a precancerous endometrial lesion; therefore, abnormal methylation of *hMLH1* is considered to be significantly involved in carcinogenesis (43).

A recent study of cullin-5 (*CUL5*) demonstrated that this gene is involved in breast and cervical cancer and hepatocellular carcinoma. *CUL5* was significantly downregulated in serous endometrial adenocarcinoma with a poor prognosis, compared with its level in endometrial carcinoma with a good prognosis (58). The action of *CUL5* involves non-coding RNAs (ncRNAs), which are RNAs that do not include genetic information for protein synthesis. These include microRNAs (miRNAs) of 18-25 nucleotides that lead to mature miRNAs that bind to the 3'-untranslated region (UTR) of target genes and inhibit gene function (46). Two highly-conserved miR-182-binding regions are present in the 3'-UTR of *CUL5*, and *CUL5* targets miR-182 in endometrial cancer. *CUL5* downregulation causes miR-182 upregulation and progression of endometrial cancer (58). Zhou *et al* demonstrated that miR-30c overexpression inhibited metastasis-associated gene-1 (*MTA1*) (59). miR-30c is decreased in endometrial, ovarian, breast and gastric cancer (60-63) and it is involved in carcinogenesis through its association with *MTA1*.

## 5. Targeting of epigenetic abnormalities in endometrial cancer therapy

Unlike genetic changes, epigenetic alterations are not irreversible, and it may be possible to use molecular-targeted drugs to induce transcription of tumor suppressor genes via demethylation (64). The effects of demethylating drugs on tumor suppression include inhibition of growth of cervical cancer cells *in vivo* by demethylation of *APC* by hydralazine (65) and 5-azacytidine-induced expression of *APC* and *hMLH1* in endometrial cancer *in vitro* (22). With regard to treatment of epigenetic changes by miRNAs, Tsuruta *et al* demonstrated *in vivo* and *in vitro* that exogenous miR-152 targeting of DNA methyltransferase 1 (DNMT1) in endometrial cancer cells without expression of miR-152 inhibited tumor proliferation (66). Zhao *et al* recently reported similar results for miR-126 targeting of insulin receptor substrate 1 (67). These results suggest that cancer therapy may be established by delivery of dsRNA.

DNMTs and histone deacetylases (HDACs) are key enzymes mediating epigenetic regulation of gene expression. The majority of events involving DNA overexpression and histone deacetylation in promoter regions are associated with transcriptional downregulation or silencing, and epigenetic silencing of tumor suppressor genes plays an important role in malignant transformation (68). DNMT inhibitors induce DNA demethylation and HDAC inhibitors cause histone acetylation, resulting in reactivation of silenced genes and functional and morphological changes in cancer cells. DNMT inhibitor-mediated demethylation of the cadherin (*CDH*)1 promoter results in upregulation of *E-cadherin* in endometrial cancer cells. A combination of DNMT and HDAC inhibitors upregulated *CDH1* and downregulated B-cell lymphoma 2 at the mRNA level, inducing cell cycle arrest and apoptosis (69). This combination has a synergistic effect and is likely to become a new treatment for endometrial cancer.

## 6. Conclusion

In uterine cancer, epigenetic and genetic changes are intertwined in a complex manner, resulting in cancer onset. Epigenetic changes cause phenomena including inhibition of apoptosis, DNA repair inhibition, overgrowth and enhanced cancer growth. Genes involved in epigenetic changes are inhibited by methylation, and reactivation of these genes may inhibit the growth of cancer cells. Epigenetic information may also be useful in screening for uterine cancer with high sensitivity and specificity and for development of novel molecular-targeted drugs, leading to improved treatment outcomes in uterine and other cancers. Therefore, further studies on cancer epigenetics are essential for improvement of cancer therapy.

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