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REVIEW ARTICLE

# Epigenetic mechanisms in endometrial cancer

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## *Summary*

***Purpose:*** *Endometrial cancer is a very common type of cancer in females worldwide. Advances in diagnosis and treatment have not decreased the incidence of endometrial cancer. Lately, research has been focused on revealing the molecular and genetic characteristics of endometrial cancer in order to provide new insights in the biology of this entity, leading hopefully to innovating therapies. Research has revealed that epigenetic modifications govern endometrial carcinogenesis. In this review, the epigenetic mechanisms that are involved in endometrial cancer as well as the differences between the different types of endometrial cancer are discussed. The review also refers to the putative therapeutic benefits that hopefully can arise.*

***Key words:*** *endometrial cancer, endometrioid, epigenetic, methylation*

## Introduction

Endometrial cancer is the fourth most common malignancy in women in Europe and the most common gynecologic malignancy in the United States [1]. The incidence of endometrial cancer has increased in the last years and despite advances in diagnosis and treatment, the death rates have steadily been increasing over the past 20 years [2]. Recent progression in research has revealed extensive epigenetic modifications that are involved in endometrial carcinogenesis and offer a window of opportunity in improved therapies. In this review, the epigenetic mechanisms involved in endometrial cancer are discussed, as well as the putative therapeutic benefits that can arise.

## Epigenetics

According to the NIH "Roadmap Epigenomics Project," the term epigenetics refers to both heritable changes in gene activity and expression (in the progeny of cells or of individuals) and also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. Epigenetic modifications are defined as any modifications in genomic DNA that do not allow transcription of DNA thus causing transcriptional silencing. These modifications are not affected by cell division, they do not alter the genome's sequence and they can be both beneficial and detrimental. In cancer, for example, epigenetic modifications keep the genome safe by not allowing rearrangements in chromatin that can cause high gene activation but can also be harmful through silencing of tumor suppressor genes. Comprehension of the epigenetic mechanisms in carcinogenesis is valuable for developing and ameliorating cancer treatment and prevention [3]. There are three different types of mechanisms that cause gene silencing: DNA methylation, histone modifications and RNA–associated silencing.

DNA methylation is now considered a hall-

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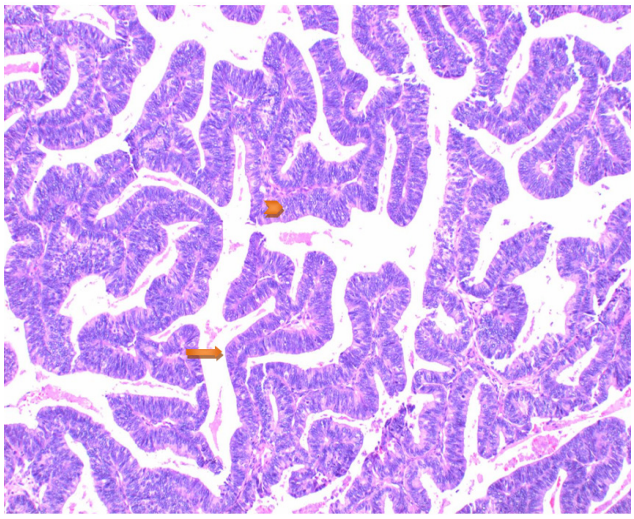

**Figure 1.** Well differentiated endometrioid adenocarcinoma (H&E x400). Glandular cells with nuclear stratification featuring minimal to moderate atypia (arrowheads). There is a sharp demarcation of the apical border of the neoplastic glands (arrows).

mark of cancer. DNA is methylated by DNA cytosine methyltransferases (DNMT1, 3A and 3B), that catalyze the transfer of a methyl group to a cytosine nucleotide next to a guanine (CpG) and 5-methyl-2'-deoxycytidine (5-mC) is formed. These CpG clusters are called CpG islands and can also be found either near or in gene promoters [4]. CpG islands in gene promoters are not methylated [4,5], therefore they allow transcription while in the rest of the genome they are heavily methylated [6]. Aberrant DNA methylation seems to happen early in endometrial tumorigenesis and it is a universal phenomenon that affects many critical genes [7].

Post translational modifications of histone proteins are another epigenetic mark. In genes where transcription is active, core histones H2A, H2B, H3 and H4 have specific acetylated lysine residues that prevent the histones from being in close contact with the DNA in the nucleosome. Thereafter, transcription factors and polymerases are free to reach coding sequences and commence transcription. Many his¬tone deacetylases (HDACs) that reverse these modifications are associated with gene silencing [8]. While acetylation occurs in euchromatin, histone methylation is also an epigenetic mark popular in heterochromatin as well as in euchromatin and it is orchestrated by histone methyltransferases and histone demethylases [9]. Differential methylation in the same histone can both be a marker of gene activation or silencing [3]. Histone deacetylation that leads to gene silencing is also associated with DNA methylation, while histone marks that activate gene transcription are also observed with DNA hypomethylation.

Another mechanism that causes gene silencing is RNA-associated silencing and is induced by micro RNAs (miRNAs) [3]. miRNAs are implicated in the development, cell cycle and cell death [10]. Moreover, it has been shown in many studies that miRNAs play an important role in cancer [11]. The most interesting finding is that miRNAs can cause histone modifications, DNA methylation, changes in chromatin status [3], as well as regulation of the expression of DNMTs and HDACs which makes them key players in cancer epigenetic regulation. miRNAs have also been identified as targets of epigenetic changes.

## Epigenetic modifications in type I endometrial cancer

Two different subtypes of endometrial cancer are recognized: type 1 or endometrioid (estrogen-related) and type 2 or non-endometrioid (non-estrogen related) (Figure 1). Eighty percent of newly diagnosed cases in the Western world are of type I and are mostly encountered in young or perimenopausal women under unopposed estrogenic stimulation [1]. These tumors (endometrioid carcinomas, EECs) resemble morphologically the normal endometrium and arise in a setting of endometrial hyperplasia [12]. They have usually minimal myometrial invasion, exhibit low-histological grade and are often cured with hysterectomy.

Promoter hypermethylation is the most common epigenetic mechanism found in EECs. Nieminen et al. identified 24 tumor suppressor genes whose promoters were progressively hypermethylated during the development of the disease. What precedes though this epigenetic modification and might be responsible for the appearance of it is microsatellite instability (MI). MI is present in 20-35% of EECs and it is hypothesized that it provokes alterations in many regulatory genes involved in DNA repair, apoptosis, transcriptional regulation and signal transduction that promote carcinogenesis [13]. The most common mechanism for tumor suppressor gene silencing in endometrial cancers with MI is MLH1 promoter hypermethylation and studies have shown that it is an early event in cancer progression [14]. Promoter hypermethylation is not only present in EECs with MI that lack MLH1 expression but also

**Table 1.** Possible epigenetic biomarkers for EEC (type I)

| *Epigenetic biomarker* | *Normal cellular role* | *Epigenetic alteration* |
|---|---|---|
| *Promoter hypermethylation* | | |
| hMLH1 | DNA mismatch repair gene | Lack of expression |
| PTEN | Tumor suppressor gene | Gene silencing |
| RASSF1 | Tumor suppressor gene | Gene silencing |
| HAND2 | Transcriptional factor expressed in endometrial stroma | Gene silencing |

in cancer cell lines that lack the mismatch repair mechanism [15]. Furthermore, the demethylation agent 5-aza-2'-deoxycytidine of the *MLH1* gene was found to initiate MLH1 expression and restore the activity of mismatch repair genes.

Besides MLH1, promoter hypermethylation has been identified in other genes such as RASSF1A, *MGMT* and *PTEN* in tumors with MI. *PTEN* is a tumor suppressor gene that can be silenced through promoter hypermethylation but also with loss of heterozygosity (LOH) and mutations. PTEN is the most common mutated gene in EECs (30–50%) [16,17], and recent studies have linked PTEN promoter methylation with advanced stage in type 1 endometrial cancer [18].

*MGMT* is another silenced DNA repair gene that is present in 48% of EECs [19]. Loss of MGMT function leads to recognition of O(6)-methylguanine as adenine by DNA polymerases. O(6)-methylguanine is a pro-mutagenic form that leads G to A mutations [20].

*RASSF1A* is a human tumor suppressor gene that acts as a negative regulator of the RAS-MAPK signaling pathway, which is frequently altered in EECs. Loss of *RASSF1A* due to epigenetic gene silencing is correlated with increased activity of the RAS-MAPK pathway. *RASSF1A* silencing through promoter hypermethylation is a very common feature of advanced stage of type 1 endometrial carcinomas (74%) and is also related to higher frequency of lymph node involvement, to higher grade tumors, to higher incidence of recurrence and to lower disease-free survival [21].

*HAND2* methylation has recently been detected in type 1 endometrial cancer. *HAND2* encodes for a transcription factor expressed in the endometrial stroma and was found to be severely hypermethylated [22]. Premalignant endometrial lesions showed enhanced *HAND2* methylation. *HAND2* methylation is a good potential biomarker for EECs, however further research is required to assess its true clinical use. The possible epigenetic biomarkers for the EECs are shown in Table 1.

## Epigenetic modifications in type II endometrial cancer

Type II or non-endometrioid carcinoma (NEEC), is diagnosed in older postmenopausal women [13] and is more frequent in African–American women [23]. These tumors are not associated with estrogen and they are mostly high-grade serous or clear cell carcinomas (Figure 2). Unlike type I tumors that are mostly confined to the uterus, type II carcinomas invade deeply into the myometrium and are characterized by early extrauterine disease; as such they have to be treated in a more aggressive manner [13]. NEECs also seem to carry different genetic alterations from EECs. They are characterized by aneuploid karyotypes, LOH and aberrant p53 mutations. *p53* alterations with simultaneous overexpression of the

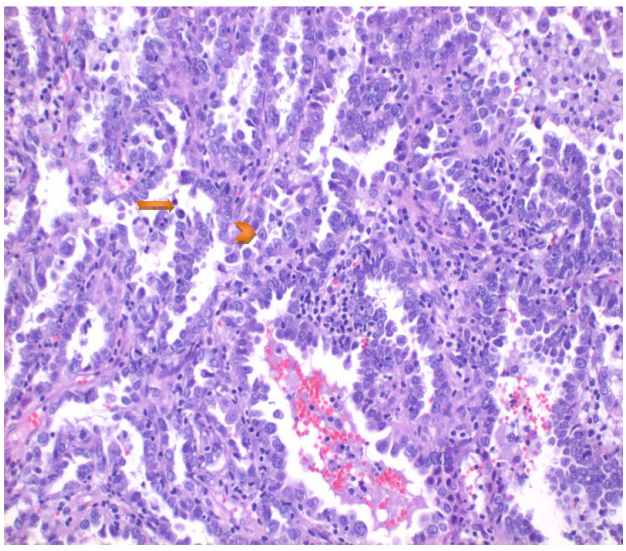

**Figure 2.** Serous endometrial cancer (H&E x400). The neoplastic cells are highly atypical with nuclear pleiomorphism and prominent nucleoli (arrow heads). There is cellular desquamation with many "free floating cells" (arrows).

**Table 2.** Genetic alterations in NEEC (type II)

| *Possible involved genes/proteins* | *Normal cellular role* | *Genetic modification* |
|---|---|---|
| p53 | Tumor suppressor gene | Inactivation |
| Cyclin D1/ Cyclin E | Regulators of CDK | Upregulation |
| Her-2/neu | Oncogene – Epidermal growth factor receptor | Upregulation |
| e-cadherin | Type-1 transmembrane protein | Reduction |
| STK15 | Putative oncogene- accurate segregation of chromosomes during mitosis | Overexpression |

PI3K/AKT pathway are also observed in NEECs [24]. The PI3K/AKT pathway is a signaling pathway that regulates cell cycle and promotes cell growth and proliferation. In many types of endometrial cancers it is constitutively active. Results from the above-mentioned study imply that *p53* inactivation and activation of the PI3K/AKT pathway in high-grade endometrial carcinomas is consistent with poor prognosis.

NEECs are also associated with Cyclin D1, Cyclin E and Her-2/neu upregulation and reduced E-cadherin gene expression [1], all of the above being cellular changes that promote cell proliferation and oppose apoptosis. They also exhibit STK15 overexpression, which is responsible for increased chromosomal instability. Promoter hypermethylation seems to play a less important role in type II cancers. Promoter hypermethylation of many genes such as *MLH1*, *PTEN*, *MGMT*, and *RASSF1A* is not detected in type II tumors [25]. Loss of progesterone expression is observed in NEECs [26,27], but it is not clear yet whether its inactivation is caused by methylation, a fact that could help in designing new hormone treatment strategies for this type of cancer in the future. DNMT1 and DNMT3B are also downregulated in NEECs [28,29]. As already mentioned, DNMTs catalyze DNA methylation and a possible scenario is that this downregulation can cause global hypomethylation in NEECs and might be the reason for the histological differences between EECs and NEECs. Nevertheless, more studies need to be conducted in order to assess the different epigenetic mechanisms that underlie the different types of endometrial carcinogenesis and design prevention approaches.

Finally there is an agreement in many studies that some NEECs might arise from preexisting EECs through dedifferentiation. These tumors would possess molecular, histopathological and immunohistochemical features from both types [12]. Most of those carcinomas fall in type II category and a small subset seems to represent type 1 cancers. Possible genetic alterations found in NEEC type II are listed in Table 2.

## Current research

There are many methods that have been used over the years in order to detect DNA methylation such as DNA sequencing [30], q-PCR [31], microarrays [32], mass spectrometry [33] and combined bisulfite restriction analysis (COBRA) [34]. Of these, the most popular method is sodium bisulfate treatment of DNA followed by single molecule sequencing that detects cytosine DNA methylation [35]. Throughout the years, with advances in technology, such as second generation sequencing, many more methods have been developed like whole-genome bisulfite sequencing [36,37], differential methylation hybridization analysis (DMH) [38] and deep single molecule bisulfate sequencing [39].

The most recent study was performed by Zhang et al. [40] who investigated DNA methylation in the two types of endometrial cancer as well as in normal tissues through methylated DNA immunoprecipitation sequencing (MeDIP-seq) and methylation-sensitive restriction enzyme digestion sequencing (MRE-seq). The aim was to identify local differentially methylated regions (DMRs) and it is the first time that a whole-genome DNA methylation map was created for endometrial cancer. Many DMRs were common in both subtypes but some were specific to each cancer subtype and some of them were different in normal endometrium. With the use of these techniques many DMRs were identified that could not be discovered with array-based platforms [40]. These DNA methylation changes seem to be an important signature of endometrial cancer and could possibly serve as biomarkers in the future. Nevertheless, the main disadvantage of the above described techniques is that, despite their wide-

spread use in research, they are not cost-effective and therefore they cannot be used for diagnostic routine use.

Besides DNA methylation, a number of miRNAs was identified to play a role in the development of endometrial cancer. Many of them are involved in processes including cell death, growth, proliferation, and carcinogenesis [41]. Balch et al. [2] performed an extensive literature research and recorded all the miRNAs that are implicated in endometrial cancer. Nonetheless, a specific distinct pattern for each type of endometrial cancer has not yet been established. As miRNAs are detected in body fluids of many cancer patients, using them as cancer biomarkers is a minimal invasive way to detect the disease. Thus, investigation of these miRNAs should become top priority in endometrial cancer biology.

## Conclusions

During the last years the important role of epigenetic modifications in carcinogenesis has become evident. One of the most interesting revelations was the impact of epigenetic modifications in endometrial carcinogenesis, from development till therapy. Understanding the mechanism by which epigenetic alterations along with genetic mutations, lifestyle and environmental factors lead to disease is the ultimate goal. Another important clinical impact in the use of epigenetics is in diagnosis with the help of emerging new technologies. New technologies can help in identifying the distinct methylation profile of each patient, thus leading to a more personalized treatment that would probably improve the patient's life.

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