Biological markers with potential clinical value in endometrial cancer — review of the literature

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ABSTRACT
Endometrial carcinoma (EC) is the most common malignancy of the female genital tract encountered in western countries, making it the fourth most common cancer in women. The incidence of uterine cancer is on the rise throughout the developed world where diagnosis is increasingly observed among younger patients. With regard to this, attention has been focused on conducting more studies to achieve a better understanding of the molecular genetics related to endometrial carcinogenesis. Over the years, EC has been classified into two broad histopathological subtypes based on the mechanism of development, and we can therefore observe specific biomarkers related to the respective subtype. Based on this idea, more research has been carried out in the last decade, using biotechnological methods, with the aim to identify new potential tumor markers. By translating these findings into clinical use one may facilitate accurate diagnosis and prognostic prediction, and contribute to individualized treatment. Without a doubt, there is a demanding need to identify biomarkers that can be adopted in clinical practice in order to reduce the time needed to obtain diagnosis. Such markers may be of great value in improving patient outcome. However, a number of problems remain to be solved before this becomes a reality. This paper briefly reviews the current status of rising biomarkers in EC.

INTRODUCTION
Endometrial cancer (EC) is the most frequent type of gynaecological malignancy in developed countries [1]. The diagnosis is commonly observed among postmenopausal women that seek medical attention following initial presentation of atypical vaginal bleeding prior, during or after menopause. Despite being one of the most common gynaecological malignancies, routine screening is not recommended as majority present with an early stage disease (stage I or II) resulting in favourable prognosis and excellent survival rate (5-year overall survival 75–90%) [2]. However, women encountering more advanced or recurrent disease will have an extremely poor clinical outcome. Thus, renewed research focus on better understanding the molecular changes associated with EC, which is mainly promoted by the dramatic increase in incidence observed in the recent years [3]. In contrast to cervical cancer, there is still insufficient evidence to recommend any cost-effective screening method in women with average to high risk and without symptom presentation [1]. However, papanicolaou (Pap) smear, tranvaginal ultrasound, and endometrial sampling are techniques under investigation for their ability to reveal EC at an early stage [4]. For the same purpose, different molecular techniques have been applied in the search for markers that can be associated with EC stage, prognosis, and therapeutic response. Therefore, the aim of this paper is to briefly outline a panel of promising biomarkers that can be adopted as serum screening in cancer detection and prediction of outcome.

BIOMARKERS RELATED TO HISTOPATHOLOGICAL SUBTYPES OF EC

Based on the mechanism of development, we distinguish between two broad clinicopathological variants. Type I (endometrioid) cancers comprise the large majority (70–80%) and is know to be estrogen-responsive. This subtype is as-
associated with unopposed estrogen stimulation and is more frequently observed among perimenopausal middle-aged women [2]. By contrast, type II (non-endometrioid cancer) follows the estrogen-unrelated pathway, which seems to arise from a background of atrophic endometrium. Type II tumors are commonly diagnosed in older postmenopausal women, and are generally less differentiated accounting for a poor prognostic outcome [2, 3]. A wide variety of proposed biomarkers have been examined for EC of the respective subtype. Defects in DNA mismatch repair genes, microsatellite instability, and mutations in the PTEN and K-ras and/or B-catenin genes are mutated in high rates for type I, whereas alteration in the p53 suppressor gene with mutation of Her-2/ neu are commonly observed in type II [2, 3]. Based on the significantly different gene expression profile, one can suspect that the two types may have distinct underlying etiologies, which in turn is responsible for the pathogenesis and progression [3]. For that reason, these biomarkers are currently used as diagnostic clues representing the most common basis for prognostic estimation of this gynaecological malignancy [2].

**ESTABLISHED MOLECULAR PROFILING TECHNIQUES**

**Array-based technology**

This is a well-established method for the investigation of gene expression in organs or tissues undergoing pathological changes [5]. Several studies have used array analysis to investigate genomic features of EC, resulting in detection of a large range of molecular alterations. To illustrate, Xue-Lian and colleagues performed oligonucleotide microarray to examine the global expression pattern of tumor-associated endothelial cells from EC [6]. The study was able to identify a consistent overexpression of certain marker genes in addition to loss of several tumor suppressor activities associated with EC.

Meanwhile, in a different article, cDNA microarray was applied in the investigation of expression profiles for genes encoding extracellular matrix (ECM) proteins by comparing level of markers in early and advanced stage of EC [7]. Initially, the authors presented an overexpression of six different ECM components shown to play an important role in the carcinogenesis of EC; aggrecan, collagen type VIII chain α1, collagen type XI chain α2, vitronectin, nidogen, and tenascin R. Gene microarray have attracted wide attention because of its ability to investigated hundreds to thousands of genes in parallel providing unique information about the expression of different genes related to EC [8]. Thus, becoming an important analytical tool in cancer research and clinical diagnostics.

**Next-generation sequencing (NGS)**

In a paper published by Creighton et al. a panel of novel miRNAs were discovered in the female reproductive organs using next-generation sequencing (NGS) technique (Tab. 1). Similarly, by performing whole-exome sequencing, Liang and colleagues were able to identified 12 potential cancer genes. However, AT-rich interactive domain 1A (ARID1A) was the gene attracting most attention because of its suggested role in suppressing cell proliferation of ovarian and endometrial cancer cell lines [9]. The recent introduction of sequencing technology have aided in the discovery of new RNA molecules while providing more detailed understanding

<table>
<thead>
<tr>
<th>miRNA Family</th>
<th>Expression Level</th>
<th>Potential Clinical Role</th>
<th>References</th>
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<tbody>
<tr>
<td>miR-200a, miR-200b, miR-9, miR-92, miR-449a</td>
<td>Dysregulation</td>
<td>FIGO staging</td>
<td>21</td>
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<tr>
<td>miR-203, miR-429</td>
<td>Dysregulation</td>
<td>Histological grading</td>
<td>21</td>
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<tr>
<td>miR-96, miR-183, miR-449a</td>
<td>Dysregulation</td>
<td>Cancer relapse</td>
<td>21</td>
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<tr>
<td>miR-203, miR-429</td>
<td>Dysregulation</td>
<td>Lymph node metastasis</td>
<td>21</td>
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Table 1. Novel miRNAs in the female reproductive organs
of biological pathways related to endometrial tumorigene-
sis [10]. Compared with traditional microarrays, which can only
detect a limited number of miRNA, NGS enables in-depth
characterization of the global range of miRNAs [10, 11].
Also, it is suggested that genomic analysis of cells collected
during Pap smears holds promise for early detection of EC
[4, 10]. Thus, genome-wide studies using NGS can provide
insight into the genomic alteration in association to EC.

Proteomics
Microarray and NGs studies have both provided unique
information related to gene expression profile in EC, how-
erver, information at a protein level is crucial in order to
include post-translational events [7]. In several papers, pro-
teomics has been used to assess the clinical utility of bio-
markers to evaluate their diagnostic and prognostic sensitiv-
ity and specificity by comparing protein profiles between
pathological and normal tissue. It is belived that proteins are
directly linked to the phenotype and the malignant nature
of cancer, which explain the increased interest for studying
global protein expression [11]. A study performed by Li et al.
exemplifies the value of proteomics where three potential
EC-associated proteins were identified: Cyclophilin A (CypA),
epidermal fatty acid-binding protein (E-FABP), and calypho-
sine (CAPS) [11]. Additionally, increase in both E-FABP and
CAPS in relation to EC were also reported in a different pa-
per [12]. Implementing this method, both papers conclude
that the overexpression of E-FABP and CAPS is correlated
to histodifferentiation but not to clinical staging [11, 12].

As identified by plasma membrane proteomics tech-
nique, an overexpression of bone marrow stromal anti-
gen 2 (BST2) was demonstrated in EC at both mRNA and
protein level. Based on this finding, it was proposed that
BST2 might have a suppressor effect on tumor growth by
either blocking the function of target signalling molecules
or receptors, or by stimulating apoptosis. For that reason,
it is suggested that BST2 might have a potential value as
a molecular therapeutic target [13]. Lastly, Maxwell et al.
performed global differential proteome to identify the
level of proteins associated with in stage I EC [14]. Interest-
ingly, an overexpression of specific ribosomal proteins (RS3,
RS9, RS14, RS18, RL1A, RL2A, RL8, RL11, RL22, RL18, RL24,
RL10A, RL27A), which has not been previously described
in acquaintance to EC, was established in this report. More
importantly, a deregulation was found to include the follow-
ing set of proteins; (1) multiple members of peroxiredoxin
family (PRDX1, PRDX3, PRDX4, PRDX5, PRDX6), (2) prohibitin 2
(PHB2), and (3) members of the annexin family (ANXA1,
ANXA2). As demonstrated by the abovementioned findings,
we can emphasise that proteomic is an innovative approach
for the identification of proteins and biomarkers that can be
clinically adopted for diagnosis of EC [14].

Biomarkers with diagnostic, prognostic and therapeutic value in EC

Astrocyte elevated gene-1 (AEG-1/MTDH/LYRIC)

While some papers are primarily focusing on further
investigation of the already known set of genetic alterations,
others are aiming to detect newer cancer genes (Tab. 2). The
comprehensive list of novel biomarkers related to EC includes
the recent discovery of Astrocyte elevated gene-1. AEG-1 is
located at chromosome 8q22 [15] and is also known as
metadherin (MTDH) and lysine-rich CEACAM1 coisolated
(LYRIC) [17]. Since its discovery in 2002, as HIV- and TNF-
α-inducible gene in primary human fetal astrocytes [15],
several authors have described its tumor-promoting activity
which is related to the activation of diverse signal transduc-
tion pathways (PI3K/ AKT, NF-κB, MEK/ERK, WNT/β-catenin)
involved in cancer progression, in addition to its role in
pathogenesis, metastasis, invasion, angiogenesis and over-
all patient survival [16]. It should be noticed that AEG-1 is
an important oncogene were its expression status is firmly
established in a subsequent array of cancers.

More importantly, AEG-1 has been described to play a
central role in carcinogenesis and progression of endo-
metrial cancer in a study conducted by Song et al. [17]. The
expression rate of AEG-1 was investigated in 35 normal
endometrial tissue, 40 atypical hyperplasia, and 174 EC
tissue (161 cases being endometrioid carcinoma) show-
ing a gradual elevation with the transition from normal to
cancerous tissue. Thus, AEG-1 was found to be significantly
correlated with clinicopathological parameters including
FIGO stage (p < 0.001), depth of myometrial invasion
(p = 0.015), lymph node metastasis (p = 0.005), lymph vas-
cular space invasion (p < 0.001), recurrence (p < 0.001), and
Ki-67 expression (p = 0.032). Several authors have implied
that an up-regulation of AEG-1 enhances characteristics
of malignant aggressiveness making it an independent
prognostic factor for unfavourable clinical outcome. This
suggests that AEG-1 is valuable as a prognostic biomarker
of disease progression and survival in patients with EC [16–18].

MicroRNAs

Since the discovery of microRNA (miRNA), several authors
have been intensively studying their role as diagnostic and
prognostic markers, and predictors of drug response. MiRNAs
have aroused wide attention because of their suggested
role as important regulators of gene expression in a broad
spectrum of diseases, including solid and hematologic mali-
nancies [18]. This is a family of small (21–22 nucleotides)
non-protein-coding RNAs responsible for messenger-RNA
(miRNA) stability and expression of proteins at a post-trans-
criptional level [18, 19]. For that reason, miRNAs has become
well-established group of markers for the development and
progression in a wide range of malignancies. In general,
miRNAs may either act as oncogenes or tumor suppressors presenting increased or decreased expression in tumor cells [19]. This alteration in miRNA expression may be involved in the initiation, cancer progression, and metastatic process in different cancer types. In the course of EC, a study done by Tsukamoto et al. suggest that miRNAs are predominantly involved in cell proliferation, differentiation, apoptosis, and carcinogenesis of endometrium [19]. Because 118 differently expressed miRNAs associated to EC have been reported so far, clinically important miRNAs that contribute in the cancer progression has to be identified [20].

During the past decade, several miRNAs including hsa-miR-503, hsa-miR-205, and hsa-miR-200b were shown to be dysregulated in endometrioid endometrial carcinomas (EEC) [19, 20]. Moreover, a comparison of EC tissue to normal tissue control detected an up-regulation of miR-200 family, which contains five miRNAs localized in two genomic clusters, chromosome 1 and 12 [20]. Corresponding with these findings Torres et al. reported a significant up-regulation of all miR-200 family members, mostly pronounced in the early phase of EEC [21]. Additionally, an over expression was found to encounter miR-205 and miR-210, suggesting them to be selected as biomarkers for the early diagnosis and prognosis of EC [22]. Xiong and colleges studied miRNAs in relation to early stage (stage I) EEC, and identified a deregulation of hsa-miR-196a-5p, hsa-miR-328-3p, hsa-miR-337-3p, and hsa-miR-181c-3p indicating their clinical value as potential diagnostic markers [18]. By comparing gene expression patterns in normal endometrium, atypical hyperplasia and EC tissue, Boren et al. described a total of 13 miRNAs that demonstrated a significance difference in level of expression [23]. In the transition from normal endometrium through atypical hyperplasia to cancer, five miRNAs (miR-let 7i, miR-221, miR-193, miR-152, miR-30c) exhibited a decrease in expression, leaving the remaining eight miRNAs (miR-185, miR-106a, miR-181a, miR-210, miR-423, miR-103, miR-107, miR-let 7c) with a relative increase in expression. Initially, there were no association between the miRNA expression and cancer stage or grade [23]. In another study aiming to investigate the clinical and pathological characteristics, a set of miRNAs was found to be dysregulated in regards to FIGO staging (miR-92a, miR-96, miR-200a, miR-203, miR-429, miR-449a), histological grade (miR-200b*, miR-429, miR-9, miR-92, miR-449a) occurrence of relapse (miR-96, miR-183, miR-449a), and lymph node metastasis (miR-203, miR-429) [21]. Additionally, an over-expression of miR-77 family was contributed to clinically more advanced tumors. The set of miRNAs presented in this paragraph were all identified by means of microarray technology and/or next-generation sequencing (NGS) with further conformation using qRT-PCR. To investigate miRNA profiles, both plasma and tissue were collected form patients with EEC revealing their potential as future noninvasive biomarkers for early detection, diagnosis and prognosis of EC [18, 21]. Finally, it is suggested that miRNA can aid as potential therapeutic target by either blocking or mimicking the miRNA activity, however, further research need to be carried out [22, 23].

<table>
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<th>Table 2. The most significant molecular markers in EC detection</th>
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<td><strong>Biomarkers</strong></td>
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<td>Extracellular matrix (ECM) proteins (aggrecan, collagen type VIII chain α1, collagen type XI chain α2, vitronectin, nidogen, tensacin R)</td>
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<tr>
<td>Epidermal fatty acid-binding protein (E-FABP)</td>
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<td>Cacyphasine (CAPS)</td>
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<td>Bone marrow stromal antigen 2 (BST2)</td>
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<td>Astrocyte elevated gene-1 (AGE-1)</td>
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<tr>
<td>Cyclophilin A (CypA)</td>
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<tr>
<td>Human epididymis protein 4 (HE4)</td>
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<td>Matriptase (MT-SP1)</td>
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Cyclophilin A (CYP A)

CypA is among the proteins that have been repeatedly reported to be involved in pathogenesis of several diseases including cancer, cardiovascular disease, and viral infections. This is a cytosolic binding protein that belongs to the immunophilin family, which are found in all prokaryotes and eukaryotes and is believed to have an important role in regulating protein folding process, T-cell activation, differentiation, cell migration, proliferation, and Bcl-2 expression in various cells [24]. Research confirms its involvement in several types of cancer, were an up-regulation is believed to be correlated with poor outcome of the patients [25]. To date, only one proteomic study on CypA has been reported which presented an overexpression of CypA in EC based on individual-matched cancer specimen and normal endometrial tissue. Among 99 proteins identified, CypA was found to be one of the most significantly overexpressed protein in all EC tissues examined. Perhaps the clinically proved up-regulation of CypA in EC may be applied as an independent predictor of survival. Its potential value as a biomarker for prognosis and clinical treatment is supported by a selection of criteria, which include involvement and overexpression in EC, significant difference between EC specimen and control tissue, and its identification by mass spectrometry [26]. However, the precise role of CypA in targeted treatment of endometrial cancer remains to be established.

Complementary to this, other studies have also aimed to investigate CypA as a potential marker in different cancer types. The results of those studies were similar and the CypA overexpression was shown to be significantly more likely to present with poor differentiation and decreased survival.

Human epididymis protein 4 (HE4)

In gynaecological malignancies, HE4 have merged as a promising biomarker and was first described by Kirchhoff et al. by means of cDNA screening. This protein is also known as Whey acidic protein (WFCD2) localized on human chromosome 20q12-13.1 and is identified as one of four cDNAs highly expressed in the epididymis, trachea, lung, prostate, endometrium and breast [27]. In 2001, the United States Food and Drug Agency (FDA) approved this protein for monitoring of recurrence and progression in epithelial ovarian cancer. Because EC possesses many similarities to ovarian cancer it was desirable to investigate HE4 and its relations to EC. Brennan et al. performed a large population-based cohort study to evaluate if serum HE4 can offer preliminary pre-operative risk stratification for EC. Specifically because of the high expression level of HE4 in EC tissue, and the increased serum level in this group of patients [28, 29]. The result strongly implied that serum HE4 was an independent poor prognostic marker, and it was suggested to use HE4 serum assay as a cost-effective approach to avoid unnecessary lymphadenectomy in patients with low risk EC. In a different paper the expression of HE4 in EC and its relations to clinicopathological parameters and prognosis of EC was studied. The goal was to detect the expression rate of HE4, by means of immunohistochemical using streptavidin-peroxidase, in EC, endometrial atypical hyperplasia, and normal endometrial tissue samples, respectively [27, 29]. The results implied that the intensity of HE4 expression increased with degree of malignancy. Thus, the level of HE4 in EC was significantly higher than that of hyperplasia and normal endometrium. Furthermore, the investigation showed no relations of HE4 to the pathological subtype but rather a strong relation to other factors like cancer stage, metastasis, myometrial invasion depth, recurrence, degree of differentiation and the overall survival rate. However, in another paper there was a lack of evidence to estimate the clinical value and to support the application of HE4 in EC [30]. In conclusion, further researches have to be carried out in order to evaluate the clinical specificity and sensitivity of HE4 and its benefits as a serum marker for EC.

Matriptase (MT-SP1)

The type II transmembrane serine protease (TTSP) family has recently gained increased interest because of their link and potential to enhance the aggressive nature of cancer cells [31]. The matriptase, a subfamily of TTSP, which is normally expressed by cells of epithelial origin, is suggested to be involved in the degradation of the extracellular matrix (ECM), including interstitial basement membrane (BM) in certain tumor entities. Therefore, high levels of matriptase will in many cases be correlated with poor clinical outcome [31, 32]. Matriptase, originally isolated from breast cancer cells, is thought to have a pleotropic function where its carcinogenetic properties are to facilitate cellular invasion and activation of oncogenic pathways [32]. Protease is functionally involved in tumor growth and spread in a variety of benign and malignant tumors where its overexpression is confirmed in a large number of studies.

Nakamura et al. studied matriptase in association to human EC assessed by immunohistochemistry for evaluation of epithelial cells [33]. The expression level was compared in normal endometrium, endometrial hyperplasia and in EC tissue respectively. The immunostaining patterns of matriptase were then classified into strong, moderate, and weak cell staining. EC showed the strongest expression in comparison to normal and endometrial hyperplasia. It was concluded that matriptase elevation was associated with clinicopathological parameters such as advanced stage, high grade, myometrial invasion depth, cervical involvement, lymph node metastasis, lymph vascular space involvement and peritoneal cytology. Strong matriptase expression is therefore linked to an overall lower survival rate [30–33].
Furthermore, matritase is showed to be effective in prevention of tumor growth and metastasis formation, which makes it both a potential new target for anti-cancer therapy as well as a novel prognostic diagnostic marker in several cancer types including EC [32].

**CONCLUSIONS**

The increase in incidence of EC raises the need for discovery of more convenient methods that may contribute to early detection and better prognostic assessment. A panel of new genes and proteins have therefore been intensively studied during the past decade. Some of which are aforementioned in this paper have aroused considerable attention making them promising in future clinical application. However, each biomarker provides only limited information and the search for biomarkers with higher sensitivity and specificity is required for screening, diagnosis, prognosis, and individualized therapy.

**Financial disclosure**

Authors have nothing to disclose.

**REFERENCES**


