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ENDOMETRIAL CANCER AND APPLICATION OF PROTEOMICS

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ENDOMETRIAL CANCER

Endometrial cancer (EC) is the fourth most common type of cancer among women and the most common gynecologic malignancy which accounts for 6% of all cancers in females. Lifetime risk in the population is about 2%. Since 1960, the number of new cases has gradually risen; EC now accounts for 40 % of gynecological cancers. The mortality rate for EC is 7–10 per 100,000 women. Obesity, hypertension, physical inactivity, diabetes, and family history are among the most important risk factors for developing EC [1, 2] . Thus, EC is one of the growing public health problems.

EC is divided into two pathological subtypes. *Type 1* endometrioid endometrial carcinomas (EECs) account for 80% of all EC, and affects pre- and perimenopausal women. Morphologically, *Type 1* is an endometrioid cancer. In most cases of *Type 1* tumors, EC is preceded by hyperplasia that is largely estrogen-dependent and caused by estrogen simulation that is not balanced by progesterone. These tumors are usually highly differentiated and have relatively good prognosis. *Type 2* non-endometrioid endometrial carcinomas (NEECs) affects older, postmenopausal women whom the non-neoplastic endometrium is atrophic. Morphologically these tumors are non-endometrioid carcinomas and are serous or clear cell tumors. *Type 2* tumors develop directly from the endometrium without hyperplasia and invade deeply into myometrium and follow aggressive clinical courses. They are often poorly differentiated and have a worse prognosis. Besides the morphological differences, *Type 1* and *Type 2* EC can also distinguished by genetic alterations [2, 3, 4] .

MOLECULAR GENETICS OF ENDOMETRIAL CANCER

Mutation of PTEN is one the frequent genetic alterations in endometroid carcinomas. Approximately 83% of endometroid carcinomas and 55% of precancerous lesions (e.g., endometrial intraepithelial neoplasia) have shown the alteration of PTEN. PTEN tumor suppressor gene is located at chromosome 10q23, encodes a protein which acts as lipid and protein phosphatase. PTEN inhibits the PI3K/AKT pathway by dephosphorylating PIP3 (product of PI3K). The protein phosphatase activity of PTEN is involved in the inhibition of focal adhesion formation, migration, and inhibition of growth factor-stimulated MAPK signaling. Decreased PTEN activity or loss-of-function mutations of PTEN causes apoptotic escape and increased cell proliferation and survival [2–4].

Other genetic changes in endometroid EC are microsatellite instability (MSIS), mutations of K-ras and β-catenin genes. Both PTEN and MSIS mutations represent an early event in endometrial carcinogenesis. PTEN, MSIS and K-Ras mutations often observed simulatenously, whereas mutations in β-catenin are usually observed alone. β-catenin plays an essential role in cell differentiation, organization of the cytoskeleton and acts as transcriptional activator in the Wnt signaling pathway [2–4]. K-Ras encodes a member of the GTPase family which is involved in signal transduction pathways between the nucleus and cell surface receptors [2, 4].

P53 mutations are the most frequent changes in *type 2* EC. The mutated p53 is a non-functional protein that often resists degradation, and leads to propagation of aberrant cells [3, 4].

Other common genetic alterations of type 2 EC are inactivation of p16, overexpression of HER-2/neu and reduced expression of E-cadherin [2,3]. Inactivation of p16 was found in 45% of serous carcinomas. p16 tumor suppressor gene encodes an inhibitor of CDK, and its inactivation leads to uncontrolled cell growth [3]. HER2/neu is an oncogene that codes for transmembrane tyrosine kinase which activates ErbB signaling network and has pro-mitogenic activity [2–4].

Model of endometrial cancer tumorigenesis has been defined by presence of these genetic alterations in endometroid (Table 1) [4].

Table 1. Pathological characteristic and genetic alteration in type 1 and type 2 ECs

Characteristic	Type I (EEC)	Type II (NEEC)
Unopposed estrogen	Yes	No
Background endometrium	hyperplastic	Atrophic
Morphology	Endometrioid	Serous, clear cell
Micro satellite instability, %	20–40	0–5
PTEN inactivation, %	50–80	10
K-ras mutations, %	15–30	0–5
B-catenin mutations, %	20–40	0–3
p53 mutations, %	10–20	80–90
HER2/neu, %	10–30	40–80
PI 6 inactivation, %	10	40
E-Cadherin, %	10–20	60–90

Table modified from Bansal N *et al.* and Doll A *et al*. [3,4]

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Abbreviations: 2-DE – two-dimensional gel electrophoresis; 2-DIGE – 2 dimensional differential in-gel electrophoresis; EC – endometrial cancer; ECCs – endometrioid endometrial carcinomas; HPLC – high-performance liquid chromatography; IEF – isoelectrofcousing; MALDI-MSI – matrix-assisted laser desorption ionization mass spectrometry imaging; MSI – mass spectrometry imaging; MSIS – microsatellite instability; NEECs – non-endometrioid endometrial carcinomas; SELDI TOF MS – surface enhanced laser desorption/ionization time-of-flight mass spectrometry.

ENDOMETRIAL CANCER: CLINICAL CONSIDERATIONS

Management of EC in clinic is a challenge, as EC ranges from diseases with good prognosis to aggressive one with poor outcome [8]. Approximately 90 % of women at the age of 60 with abnormal vaginal bleeding are diagnosed with EC [7]. The cornerstone of EC management is surgical treatment, including complete hysterectomy, bilateral salpingo-oophorectomy, and an appropriate surgical staging. In the case of aggressive tumors or advanced disease, surgical interventions have to be extended to pelvic and para-aortic lymphadenectomy and omentectomy, and adjuvant therapy is necessary for patients at high risk of recurrence [5, 7]. Postoperative treatment may be extended to radiation therapy and sometimes to chemotherapy.

However, side-effects from lymphadenectomy include lymphedema, symptomatic lymphocysts, deep vein thrombosis and blood transfusion, whereas side effects from whole pelvic irradiation include deleterious effects on the small and large intestine, urinary bladder and vaginal function [5]. Thus, pursuing optimal care for women with endometrial cancer, the intent is to avoid overtreatment and undertreatment by balancing giving adequate therapy while trying to minimize treatment side effects [5, 8].

Although the survival rates for patients diagnosed with and treated for early stage of EC is good, the prognosis of women with advanced stage disease or recurrence is poor (Table 2).

Table 2. Overall survival rates by stage for Type 1 and Type 2 ECs

Endometrioid		Non-Endometrioid	
Present in earlier stage, %		Present with advanced stage, %	
Stage I	73	Stage I	54
Stage II	11	Stage II	8
Stage III	13	Stage III	22
Stage IV	3	Stage IV	16
5 year survival rates, %		5 year survival rates, %	
Stage I	85–90	Stage I	60
Stage II	70	Stage II	50
Stage III	40–50	Stage III	20
Stage IV	15–20	Stage IV	5–10

Table modified from P.A. Gehrig, V.L. Bae-Jump [9]

For those women with early stage disease, the curative therapy is surgery with individualized use of volume directed radiotherapy. There is no efficient treatment for women with advanced stage disease. Commonly these women are treated with combination of surgery, chemotherapy and radiation. In the case of advanced or recurrent disease, when the surgical treatment is not successful, the main therapy has been chemotherapy [9].

However, a patient's risk status can be definitively determined only postoperatively. Since certain pathological factors are unavailable or inaccurate, preoperatively or intraoperatively, it may be difficult to determine which patients to select for lymphadenectomy. Preoperative histology obtained from endometrial biopsy often differs from final pathology [6]. Imaging techniques like CT, MRI and PET have poor sensitivity and specificity in detecting the depth of myometrial invasion, cervical and parametral involvement and lymph node metastasis. Therefore understanding of the molecular mechanisms of tumorigenesis may help to identify molecular markers of EC to customize both operative and postoperative treatment [6, 8].

PROTEOMICS OF ENDOMETRIAL CANCER

Discovery of new markers of EC is crucial to clinicians, since early detection of the disease and prognosis/diagnosis and monitoring of therapy can increase overall survival and cure rates of the patients. During the past two decades, combinations of high-throughput technologies have exhibited great potential in large-scale studies for biomarker discovery [11]. Besides more effective clinical therapies and marker-based screening programs, biomarker identification enables diagnosis of the disease at early stages before progression to advanced stages [11].

One of the methods of the search for new cancer markers is analysis of the expression of tumor proteins by proteomics. Proteins are the most common targets for diagnosis and treatment of cancer, since proteins provide enzymatic reactions, energy production, and transmission of information [11].

Proteomics allows comparison of hundreds or thousands of proteins to identify disease-specific biomarkers. Identification of cancer-related proteins is possible because of developments in better sample preparation techniques, protein separation, mass spectrometry (MS)-based identification, and better systemic analysis of proteomics data [10].

The most frequent proteomics methods used in studies of endometrial diseases include "gel-based" proteomics such as two-dimensional gel electrophoresis (2-DE) or differential in-gel electrophoresis (DIGE). Another technique frequently used in this field is surface enhanced laser desorption/ionization time-of-flight MS (SELDI TOF MS). These techniques have been applied with the aim of identifying expressional changes in proteins between normal and disease states, or explore molecular mechanism involved in tumorigenesis of EC (Figure). Other improved but limited in context "gel-free" proteomics-base methods are multidimensional protein separation technology, quantitative MS-based proteomics and matrix-assisted laser desorption ionization MS imaging (MALDI-MSI) [10].

The uses of proteomics-based methods opened possibility to build comprehensive proteome profiles of patients, and study expression of individual proteins. This allows to identify differentially expressed proteins that may serve as markers of EC. Performing proteome profiling for individual patients opens the possibility of personalized treatment [11].

Several proteomics-based studies of EC have been reported. In study by Yi *et al.*, role of a COX-2 inhibitor on the protein expression in RL95–2 EC cell line was evaluated, and showed that expression of COX-2 plays an important role in tumorigenesis of EC. The authors showed that COX-2 inhibitor NS-398 inhibited proliferation, viability and invasion of RL95–2 cells. This

study also identified phosphatidylethanolamine binding protein (PEBP) as upregulated, and heterogeneous nuclear ribonucleoprotein K (hnRNP K), α enolase, heat shock 70 kDa protein (Hsp70), tropomyosin and protein disulfide isomerase (PDI) as downregulated upon treatment with the inhibitor [12].

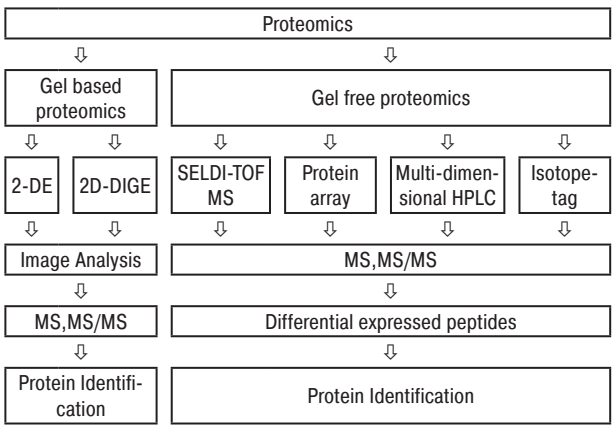

Figure. Proteomics strategies used in studies of EC: Gel-based proteomics *versus* gel-free proteomics. Gel based proteomics derives from a traditional protein separation method, such as isoelectrofocusing (IEF) and SDS-PAGE, and is based on studies of proteins. Gel-free proteomics includes arrays and mass spectrometry technologies. MS has progressed to automated and high-throughput analyses, but is limited to studies of peptides or digested protein mixtures. Figure modified from H Kuruma *et al.* [20]

Monge *et al.* analyzed role of the protein ERM/ETV5 in myometrial invasion by evaluation of those proteins whose expression was changed in endometrial cell lines overexpressing ERM/ETV5 [13]. Pathway analysis of differentially expressed proteins pointed to actin regulation, transforming growth factor-β and progesterone signaling as processes regulated by ERM/ETV5. Previously have been reported correlations between deep myometrial invasion and more undifferentiated tumors, lyph and vascular invasion, node involvement and decreased global survival. The study by Monge et al. demonstrated that ERM/ETV5 acts with involving matrix metalloproteinase-2 to provide the migratory and invasive capabilities associated with the switch to myometrial infiltration [13].

Investigation of the co-expression of survivin, c-erbB2, and COX-2 in endometrial cancer tissues, and evaluation of its prognostic significance in endometrial cancer was done in study by Lambropoulou *et al.* Co-expression score of c-erbB2, COX-2, and survivin in endometrial cancer tissues correlates significantly with classical clinico-pathological parameters and most importantly to the survival rate of endometrial cancer patients. Obtained data helped in understanding of the tumorigenesis in EC, and application of therapeutic strategies such as Cox-2 inhibition or silencing of survivin [14].

Maxwell *et al*. [18] described proteome analysis of stage I endometrial cancer tissue, and identified proteins associated with oxidative processes and inflammation. Changes in oxidative processes indicate mitochondrial dysfunction that is one the hallmarks of carcinogenesis. A number of evidences show significant increases in acidosis and hypoxia in the tissue microenvironment even at the earliest stages of carcinomas. One of the requirements of tumor cells for progression and proliferation is to acquire and maintain increased levels of hypoxia and acidosis. Identified by Maxwell et al. [18] overexpressed peroxiredoxin family proteins support this notion.

Many novel markers, such as CD171, PTEN, urokinase plasminogen activator receptor have been identified and applied in diagnostic and prognostic of EC. Other potential markers include Cyclophilin A that is involved in early carcinogenesis but not in advanced invasion or metastasis. Epidermal fatty acid-binding protein (E-FABP) contributes to carcinogenesis and metastasis by regulating differentiation and cell growth. Calcyphosine (CAPS) is a major phosphorylated substrate of cAMP-depentent protein kinase involved in the coordination of cell proliferation and differentiation [15–19]. Thus, proteomics studies delivered a number of potential markers that may improve diagnostic and prognostic of EC. However, application of proteomics to EC has only been started, and more potential markers are expected.

FUTURE PERSPECTIVE

Oncoproteomics is the growing fields which aimed to explore the mechanisms involved in carcinogenesis. In recent years, discovery of cancer markers by application of proteomic approaches has been a fast developing area. Identification of biomarkers with high sensitivity and specificity would reduce the incidence and mortality rate from EC, promote screening and enable better choice of treatment for EC patients [11].

Attempts have been made to gain insights into endometrial tumorigenesis via studies of proteins which change their expression in tumors as compared to normal endometrium. Accurate validation of these potential biomarkers into clinically relevant, diagnostic and prognostic tools is needed. Analysis of large number of samples and adequate statistical testing are essential for further progress [10].

Despite many EC-related proteins have been identified, proteomics of endometrial cancers is poorly developed. Currently, no biomarker exists in clinical practice for the detection of early stage or aggressive EC. Diagnosis therefore relies on the presence of general symptoms, which are not present in all patients. The expectation is that expansion of proteomics studies of EC will provide sufficient number of EC-related proteins to build proteome signatures for clinical use.

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