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Biomarkers as prognostic factors in endometrial cancer

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Abstract: Endometrial cancer is the most common gynecologic malignancy in more developed countries. Approximately 75% of cases are diagnosed at an early stage with a tumor confined to the uterine corpus. Although most patients are cured by surgery alone, about 15-20% with no signs of locally advanced or metastatic disease at primary treatment recurs, with limited responsiveness to systemic therapy. The most common basis for determining the risk of recurrent disease has been classification of endometrial cancers into two subtypes. Type I, associated with a good prognosis and endometrioid histology and type II, associated with a poor prognosis and non-endometrioid histology. This review will focus primarily on the molecular biomarkers that have supported the dualistic model of endometrial carcinoma and help determine which patients would benefit from either adjuvant therapy or more aggressive primary treatment.

Key words: Endometrial cancer, biomarker, prognosis.

Introduction

Cancer of the uterus is the seventh most commonly diagnosed cancer that occurs in women, with 189,000 new cases and 45,000 deaths occurring worldwide each year. About 60% of these occur in more developed countries. The highest incidence rates are in the USA and Canada. The age-adjusted incidence rate in the USA was 23.3 per 100,000 women per year [1]. In other regions, with age-standardized rates in excess of 10 per 100,000 include Europe, Australia and New Zealand, the southern part of South America, and the Pacific Island nations. Low rates occur in Africa (Uganda 3.3 per 100,000) and Asia (China 3.8 per 100,000) [2]. In Poland, the age-adjusted incidence was 13.7 per 100,000 women per year [3].

Etiology and risk factors

Endometrial cancers are known to be heterogeneous with respect to the expression of biochemical markers, ploidy, degree of differentiation, immunogenicity [4]. The disease is rare before the age of 40, and <20% occur before menopause [5]. Around 5-10% of endometrial carcinomas have a hereditary basis, with hereditary non-polyposis colorectal cancer being the most common cause [6]. The risk factors relate to hormonal stimulation of the endometrium, such as unopposed estrogen treatment, polycystic ovarian disease and estrogen-producing tumors. Overweight is present in 50% with endometrial carcinoma, and the risk is linked to disturbances in hormone metabolism related to obesity. Nulliparity is a risk factor for endometrial cancer, and breast cancer patients treated with tamoxifen have a six- to eight-fold increased risk [4,7].

Tumor biology and biomarkers

The endometrium undergoes structural modification and changes in specialised cells in response to fluctuations of estrogen and progesterone during the menstrual cycle. Long-lasting unopposed estrogen exposure leads to endometrial hyperplasia, which increases the chance of development of type I endometrial cancer. The molecular basis of this process is still not known, since the involvement of only a minority of factors is reproducible [8-11]. The development of endometrial cancer is characterized also by self-sufficiency in growth signals, insensitivity to growth inhibition, apoptosis, angiogenesis, invasion and metastasis [12].

Oncogenes

Activation of proto-oncogenes is a feature of many malignancies and, not surprisingly, there have been numerous searches for oncogene mutations in endometrial cancer [13]. The proto-oncogenes, *HER-2/neu* and *EGFR*, both members of the epidermal growth factor

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receptor tyrosine kinase (RTK) family, have an important role in regulating cell growth and differentiation, although it is not clear how a specific signalling pathway corresponds to biological and clinical response [14]. HER-2/neu overexpression is reported in 9-30% endometrial cancers and has been associated with a metastatic phenotype and poor survival in type II endometrial cancer [15]. *KRAS* encodes a member protein of the small GTPase superfamily and is involved in signal transduction pathways between cell surface receptors and the nucleus. *KRAS* mutations have been found in 10-30% of endometrial carcinomas, predominantly in type I tumors [8,9,16,17]. The *PIK3CA* (p110α catalytic subunit of *PI3K*) gene locates on chromosome 3q26.32. Phosphatidylinositol-3-kinase (PI3K) is heterodimeric lipid kinase consisting of a catalytic subunit (p110) and a regulatory subunit (p85) in PI3K/AKT signaling pathway. This pathway is frequently activated in endometrial carcinoma through various genetic alterations and their combinations. Activation of *PI3K* produces the second messenger PIP3 which subsequently activates various down-stream pathways such as AKT [4,16]. Mutations in *PIK3CA* occur in 24-36% endometrial cancers and are coexisting with PTEN mutations in 14-26% [18]. Although molecularly targeted therapies have been effective in some cancer types, no targeted therapy is approved for use in endometrial cancer. The recent identification of activating mutations in fibroblast growth factor receptor 2 (FGFR2) in endometrial tumors has generated a new avenue for the development of targeted therapeutic agents. The majority of the mutations identified are identical to germline mutations in *FGFR2* and *FGFR3* [19]. Mutations that predominantly occur in the type I of endometrial cancer, are mutually exclusive with *KRAS* mutation [8,17,20].

Tumor suppressor gene defects

A number of tumor suppressor genes have been shown to contribute to the genesis of endometrial cancers. The genes code for proteins inhibiting tumor growth. When mutated, they become inactive and growth is allowed [21]. Endometrial cancers, from a molecular viewpoint also, resemble proliferative rather than secretory endometrium. *PTEN* encodes a phosphatase, antagonizing the PI3K/AKT pathway. Decreased *PTEN* activity causes increased cell proliferation, cell survival and angiogenesis, and is involved in cell adhesion and migration. *PTEN* can be inactivated by mutations, deletions or promoter hypermethylation. *PTEN* mutations are reported in 25-83% of endometrial cancers, more frequently in type I [12,22]. The *P53* gene regulates cell cycle, apoptosis and differentiation. *P53* mutations have been found in 10-20% of endometrial carcinomas, while p53 protein overexpression is present in 15-30%. Overexpression of p53 is more frequent in type II than type I tumors [11,37,38]. *P53* mutations are considered to be an early event in type II tumors (80-90%) and a late event for type I tumors (5-10%) [8,16,22]. Alterations of the *CDKN2A* (*p16*) gene have been involved in tumor development in several organs. The *CDKN2A* gene encodes 2 proteins, p16 and ARF. The p16 protein encoded by the *CDKN2A* gene has been identified as a tumor suppressor. In endometrial carcinoma, loss of protein expression varies from 14 to 74% in different studies. The underlying mechanism of pl6 inactivation in these tumors is unknown, because mutations, deletions and promoter methylation only occur in 2-6% [23]. Loss of p16 expression is correlated with *KRAS* and *P53* mutations and is associated with high stage, high grade, and poor survival [13].

Mismatch repair genes (MMR) and microsatellite instability (MSI)

Microsatellite instability (MSI) is a condition manifested by damaged DNA because of defects in normal DNA repair process. Mammalian mismatch repair (*MMR*) genes encode for nine proteins (MLH1, MLH3, PMS1,PMS2, MSH2, MSH3, MSH4, MSH5, and MSH6) that interact with each other to form complexes and heterodimers that mediate distinct functions in MMR-related system. This repair process plays a central role in promoting genetic stability by repairing DNA replication errors, inhibiting recombination between non-identical DNA sequences and participating in responses to DNA damage [16,24]. MSI has later been found in 20-30% of endometrial carcinomas, due to epigenetic inactivation through hypermethylation of the MLHl promoter in most cases. MSI has been reported to be more common in type I than type II cancers [4,25].

DNA ploidy

Approximately 67% of type I endometrial carcinomas are diploid, as evaluated by flow cytometry. In contrast, 55% of the type II carcinomas exhibit nondiploid DNA patterns. Diploid tumors are usually low-grade type I carcinomas with only superficial invasion, and are associated with longer survival than aneuploid carcinomas. Differences in disease-free survival for stage I tumors have been as significant as 94% for diploid carcinomas versus 64% for aneuploid carcinomas. Aneuploid tumors are present in 20-35% of endometrial carcinomas, associated with a high stage, a high grade, type II and deep myometrial invasion [26,27].

Steroid receptors

The presence of the classic steroid receptors ERα and PR-A have correlated with stage, grade and survival in several studies. Additionally, it is thought that the ER and

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PR status constitutes independent prognostic factors [28]. However, PR, in contrast to ER, is suggested to be a more predictive factor of disease-free survival [29], while some authors have also reported that the presence of steroid receptors does not constitute an independent prognostic factor for endometrial cancer [30]. Therefore, the usefulness of the determination of receptor status in endometrial cancer patients is still controversially discussed.

Angiogenesis

Angiogenesis has been shown to be a critical aspect of endometrial cancer growth and metastasis [31]. The induction of angiogenesis by a tumor is a controlled process, influenced by angiogenic and angiostatic factors which involves a complex interaction between tumor and endothelial cells. Among many reported angiogenic factors, vascular endothelial growth factor (VEGF) is the most powerful endothelial-cell-specific mitogen that plays a key role in the complicated process of angiogenesis [32]. VEGF strong positive tumors showed a poorer prognosis than VEGF negative tumors. There was a trend towards an association between the strong positive expression of VEGF and 5-year disease-free survival. These results suggest that VEGF may be an important, clinically relevant, inducer of angiogenesis in type I endometrial cancer [33]. Lymphangiogenic factors have recently been studied in endometrial carcinoma, associating aggressive phenotype with the presence of lymphatic vessel infiltration and high lymphatic vessel density [34,35].

Invasion and metastasis

Invasion and metastasis are the most insidious and life-threatening aspects of cancer. Tumors of comparable size and histology can have widely divergent metastatic potential, depending on their genotype and local environmental influences. Metastatic potential is influenced by the local microenvironment, angiogenesis, stroma-tumor interactions, and elaboration of cytokines by the local tissue, and more significantly by its molecular phenotype [36,37]. Specific genetic alterations in cellular adhesion molecules, among them the cadherins and catenins, are important for such tumor-stroma and tumor-vascular interactions [38]. Changes in cadherin expression, also termed the "cadherin switch", have been associated with type II endometrial cancer [39]. Mutations in the *β-catenin* and *connexin 26* gene lead to high protein expression, and were more common in type I tumors [40,41].

Cancer stem cells and endometrial cancer

Many features of carcinoma can be explained by the stem cell concept, including clonal origin and heterogeneity of tumors, some associated with transit amplifying (TA) cells or progenitors, the mesenchymal influence on cancer behaviour, the local formation of precancerous lesions and the plasticity of tumor cells [42]. Only a small proportion of the tumor actually comprises cancer stem cells about 0.02-1%. Thus, cancer stem cells act as precursor cells that produce the proliferating, more differentiated cancer cells killed by chemotherapy or radiation. Cancer stem cells differ from normal tissue stem cells in that their proliferation is no longer controlled by the neighbouring cells of the stem cell niche [43]. As endometrial stem/progenitors cells become better characterized, their role in endometrial cancer can be assessed [44].

Dualistic model of endometrial carcinoma

Bokhman first described the pathogenetic classification of two different types of endometrial carcinoma, designated as type I and type II carcinomas, according to the determination of biological properties of the tumor, its clinical course, and the prognosis of the disease [45]. Molecular profiling reveals genetic changes in endometrial cancer that support the dualistic model, in which type I accounts for around 80%, usually of endometrioid type, estrogen-dependent, low grade lesions and type II non-estrogen dependent, poorly differentiated, not associated with estrogenic risk factors and with a poor prognosis. The molecular changes in type I tumors include mutations in *PTEN*, *PIK3CA*, *KRAS*, and *β-catenin*, along with microsatellite instability, whereas type II are characterized by genetic alterations in P53, *HER2/neu*, *p16*, and *E-cadherin*. In addition, *P53* mutations may play an important role in tumorigenesis of type II tumor. These molecular changes can help in the diagnosis of endometrial neoplasms, as well as form the basis of molecular targeted therapy [8,9,10,16,17].

Conclusions

Newer tumor markers and prognostic indicators are needed to help determine which patients would benefit from either adjuvant treatment or more aggressive primary treatment. Type I cancers are associated with mutations in *KRAS*, *PIK3CA* and *β-catenin* oncogenes, *PTEN* tumor suppressor gene and defects in DNA mismatch repair; by contrast, those of type II are associated with mutations in *P53* gene, *E-cadherin* tumor suppressor gene, HER-2/neu and p16 expression and most are non-diploid. The clinical value of these markers for prognostication in a routine diagnostic setting, and predicting response to targeted treatment, remains to be settled.

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