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Tumor Heterogeneity at Protein Level as an Independent Prognostic Factor in Endometrial Cancer[1]

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Abstract

Intratumor heterogeneity implies heterogeneous protein function, facilitating tumor adaptation which results in therapeutic failure. We hypothesized that tumor heterogeneity at protein level may influence the course of the disease. As a single biopsy might not represent the full biologic complexity of the tumor, we have analyzed immunohistochemically four different cores obtained from each primary tumor within the cohort of 364 patients with endometrial cancer (EC). The following proteins were examined: estrogen receptor 1 (ESR1), progesterone receptor, epidermal growth factor receptor, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, receptor tyrosine-protein kinase erbB-3, v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4, phosphatidylinositol-4,5-bisphosphate 3-kinase, phosphorylated v-akt murine thymoma viral oncogene homolog 1, v-myc avian myelocytomatosis viral oncogene homolog, DNA topoisomerase II alpha 170 kDa (TOP2A), cyclin-dependent kinase inhibitor 2A (CDKN2A), tumor protein p53, RAD21 homolog, S. pombe, and runt-related transcription factor 1. Particularly strong correlation was found between TOP2A and CDKN2A heterogeneity and higher stage of the disease ($P = .0002$ and $P = .0003$, respectively). Most correlations with clinicopathologic data were observed for ESR1 heterogeneity that correlated with non-endometrioid carcinomas ($P=.02$), higher stage ($P=.005$), grade ($P=.01$), and the presence of metastases ($P = .01$). Thirty-nine (11.0%) patients were classified as "globally heterogeneous". Cumulative tumor heterogeneity strongly correlated with the presence of metastases, higher stage, and higher grade of the disease (all P b .05). It also carried negative prognostic value ($P=.0008$). We show that the degree of heterogeneity in EC might serve as a clinically valid molecular marker.

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[1] This article refers to supplementary materials, which are designated by Tables W1–W4 and Figures W1 and W2 and are available online at www.transonc.com.

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Statement of author contributions: S.L.-S., W.B., and D.W. acquired the specimen material. A.S., A.J.Z., and S.L.-S. conceived the study design. H.M., J.G., and A.S. carried out the experiments. A.S. and A.J.Z. analyzed the data. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Introduction

Endometrial cancer (EC) is the most frequent malignancy of the female genital tract in the Western world, with approximately 90,000 new cases registered each year in the European Union [1]. Despite the high prevalence, the understanding of the molecular background of EC with regard to its pathogenesis and disease progression remains insufficient. Data concerning tumor heterogeneity in EC are especially scarce. Recent discoveries have shown that tumor composition is heterogeneous and consists of various cell clones. This intratumor heterogeneity depends on heterogeneous protein function, which can facilitate tumor adaptation, resulting in therapeutic failure through Darwinian selection [2]. Furthermore, intratumor heterogeneity was detected in all types of studied cancers [3,4] and may lead to more aggressive tumor behavior and unfavorable outcome [5,6].

As a single biopsy might not represent the full biologic complexity of the tumor, we used immunohistochemistry (IHC) to analyze four different cores obtained from each primary tumor within the cohort of patients with EC. Tumor heterogeneity might affect the response to treatment. Thus, the study included the expression analysis of the proteins often related to target therapies. The following proteins were examined: estrogen receptor 1 (ESR1), progesterone receptor (PGR), epidermal growth factor receptor (ERBB1), v-erb-b2 erythroblastic leukemia viral oncogene homolog 2(ERBB2, also known as *HER2*), receptor tyrosine-protein kinase erbB-3 (ERBB3), v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (ERBB4), phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA), phosphorylated v-akt murine thymoma viral oncogene homolog 1 (pAKT1), v-myc avian myelocytomatosis viral oncogene homolog (MYC), DNA topoisomerase II alpha, 170 kDa (TOP2A), cyclin-dependent kinase inhibitor 2A (CDKN2A, also known as p16), tumor protein p53 (TP53), RAD21 (RAD21 homolog, *S. pombe*), and runt-related transcription factor 1 (RUNX1). We hypothesized that the existence of cellular heterogeneity within the tumor, identified in four different cores (analyzed by IHC) belonging to the same patient, may influence the course of the disease and affect patients' survival.

Patients and Methods

Patients and Tissues

The study included 364 formalin-fixed paraffin-embedded (FFPE) primary tumor samples retrospectively collected from a cohort of EC patients who were operated in the Department of Gynaecology, Gynaecological Oncology and Gynaecological Endocrinology, Medical University of Gdańsk (Gdańsk, Poland) between 2000 and 2010. Each patient was primarily treated by surgery, with the possible option of radiotherapy and/or chemotherapy administration. The inclusion criteria were operable EC (stage IVB patients underwent cytoreductive surgery) confirmed by histologic examination and a signed consent form. The study was accepted by the Independent Ethics Committee of the Medical University of Gdańsk (NKEBN/269/2009, date: 14 September 2009). Procedures involving human subjects were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

The tumor samples included all stages of endometrial carcinoma, from stage IA to IVB, as distinguished by the International Federation of Gynecology and Obstetrics (FIGO) in 2009 [7]. We analyzed all primary carcinomas of the uterine corpus, separating them into endometrioid and non-endometrioid tumors. The latter included serous, clear cell, mucinous, mixed, squamous cell, and undifferentiated carcinomas [8]. Metastases included lymph node and distant metastases. The patients' characteristics are summarized in Table 1. The median age was 63 (range, 26-89 years). Patients with a body mass index higher than 30 were classified as obese [9]. A survival analysis was performed for 362 (99.5%) patients. After a median follow-up of 72.5 months (range, 0-158), 107 (29.4%) patients had died. The last follow-up data were collected in September 2013. The study was performed in accordance with the REcommendations for Tumor MARKer Prognostic Studies (REMARK) criteria [10].

IHC on Tissue Microarrays

Samples were collected by surgical excision before any systemic treatment and were fixed in 10% (vol/vol) neutral buffered formalin for up to 24 hours, dehydrated in 70% ethanol, and embedded in paraffin. FFPE tissue blocks were stored at room temperature for up to 14 years. The percentage of tumor cells in each FFPE specimen was evaluated by hematoxylin and eosin staining reviewed by a certified pathologist. Tissue microarrays (TMAs) were constructed from FFPE surgical resection tumor specimens and control samples. Four 1.5-mm-diameter cores from each tumor were obtained from the most representative areas (well-preserved fragments of invasive carcinoma, without necrosis, autolysis, and squamous metaplasia) using a tissue-arraying instrument (MTA-I; Beecher Instruments, Sun Prairie, WI), and then reembedded in microarray blocks.

Table 1. EC Patients' Characteristics ($N = 364$)

Variable	Number of Cases (%)
Menopausal status	
Premenopausal	27 (7.4%)
Perimenopausal	22 (6.0%)
Postmenopausal	314 (86.3%)
Missing data	1 (0.3%)
Age	
≤50 years	39 (10.7%)
>50 years	325 (89.3%)
Obesity	
Absent	173 (47.5%)
Present	190 (52.2%)
Missing data	1 (0.3%)
Histology	
Endometrioid	332 (91.2%)
Non-endometrioid	27 (7.4%)
Missing data	5 (1.4%)
Stage (FIGO)	
IA-IB	246 (67.6%)
II	53 (14.6%)
IIIA-IIIC	44 (12.1%)
IVA-IVB	16 (4.4%)
Missing data	5 (1.4%)
Grade	
I	173 (47.5%)
II	134 (36.8%)
III	47 (12.9%)
Missing data	10 (2.7%)
Cervical invasion	
Absent	268 (73.6%)
Present	91 (25.0%)
Missing data	5 (1.4%)
Myometrial infiltration	
≤1/2	168 (46.2%)
>1/2	191 (52.3%)
Missing data	5 (1.4%)
Metastases	
Absent	321 (88.2%)
Present	34 (9.3%)
Missing data	9 (2.5%)