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Original Research Article

Expression of tumor suppressor genes related to the cell cycle in endometrial cancer patients

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ABSTRACT

Purpose: Endometrial cancer is the most common gynecological malignancy in developed countries. The role of tumor suppressor genes (TSG) in endometrioid endometrial *adenocarcinoma* (EEC) has an important impact on patient survival prognosis. Thus, it is important to identify TSG transcripts that differentiate endometrial *adenocarcinoma* into various pathomorphological grades. The aim of this study was to analyze the expression profile of tumor suppressor genes related to the cell cycle in patients with endometrial *adenocarcinoma* across histological differentiation and to identify transcripts which differentiate endometrium into various pathomorphological grades.
Material and methods: Gene expression analysis was completed for 19 endometrial endometrioid *adenocarcinomas* and 5 normal specimens (obtained from women with diagnosed uterine fibroids, benign ovarian tumors and a prolapsed uterus with histopathologically confirmed endometrium in the proliferative phase) using Affymetrix HG-U133A oligonucleotide microarrays. The statistical analysis was performed using the GeneSpring13.0 software and PANTHER classification system.
Results: Significant changes in gene expression were observed across histological differentiation. The *WT-1*, *CYR 61*, *TSPYL5* genes were statistically and biologically significant in all cancer grades, and were considered to be primary for the G1 grade in endometrial cancer. The G2 cancer specific genes were *BCL2L2* and *HNRNPA0*, whereas in G3 there was only *BAK*.
Conclusion: In conclusion, the *WT-1*, *CYR61* and *TSPYL5* gene expressions are potentially correlated with patient survival in all endometrial cancer grades. The TSGs identified are considered to be important in EEC pathogenesis and further research is needed to confirm this.

1. Introduction

The increased incidence of endometrial cancer in the last decade has placed it as the most common gynecological malignancy in developed countries [1]. This trend is becoming more marked in Poland [2]. The well-acknowledged dualistic carcinogenesis model based on clinicopathological characteristics has led to two types of endometrial cancer being distinguished [3]. Approximately 75–80% of uterus malignancies are estrogen-dependent type I endometrial cancer with endometrioid morphology (EEC, endometrioid endometrial cancer) including *adenocarcinomas*. Endometrioid cancer arises from complex atypical endometrial hyperplasia and is pathogenetically associated with unopposed estrogenic stimulation. It occurs in peri- and postmenopausal women and has a good prognosis [4]. Type II cancer is characterized by non-endometrioid histology and non-estrogen dependency. It develops from atrophic endometrium and carries a poor prognosis [3,4]. The existence of two different cancer types has been confirmed by molecular biology based studies [5].

Tumor suppressor genes (TSGs) are the guardian genes that prevent oncogenic transformation. These genes play a critical role in controlling the cell cycle checkpoints that are needed for the normal outcome of proliferation and differentiation. Hence, TSGs can prevent accumulating mutations and protect the cell from acquiring cancer phenotype by inducing apoptosis [6,7]. The role of tumor suppressor genes in estrogen dependent endometrial cancer is important and has an impact on new therapies. Furthermore, gene expression changes in TSGs (p53, PTEN) are considered to be poor prognostic factors [8,9].

The aim of this study was to analyze the expression profile of tumor suppressor genes related to the cell cycle in patients with

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endometrial *adenocarcinoma* across histological differentiation and to identify transcripts differentiating endometrium into various pathomorphological grades.

2. Patient and methods

2.1. Patient characteristics

We studied 56 endometrial samples obtained from women treated at the Department of Gynecology, Obstetrics and Oncologic Gynecology, at the Medical University of Silesia in Katowice, Poland, between years 2010 and 2012. All women underwent abdominal or vaginal hysterectomy. The study group consisted of 19 endometrial specimens with histopathologically confirmed *adenocarcinoma endometrioides*. Clinically the tumors were classified according to the FIGO criteria. All patients with endometrial cancer had primary cancers and did not receive chemotherapy or radiation therapy prior to surgery. The reference group comprised of endometrial samples obtained from women with diagnosed uterine fibroids, benign ovarian tumors or prolapsed uterus with histopathologically confirmed endometrium in the proliferative phase. We excluded patients with hormone therapy for the past 12 months, severe obesity (BMI $>$ 30), endometriosis or adenomyosis, non-endometrioid endometrial cancer, adenocarcinoma with squamous elements, coexisting cervical cancer. The clinical characteristic of patients enrolled in molecular analysis is presented in Table 1.

2.2. Sample classification and storage

All analyzed tissues were collected after cutting the uterus in its sagittal plane, following the removal of the uterus *via* laparotomy or the vaginal way. The tissue samples (each approximately 1 cm) obtained were divided into two parts and placed separately in buffered formalin for histopathological studies and RNA later solution (Life Technologies, Carlsbad, USA) for molecular analysis according to the producer's instructions. Histological examination was performed according to WHO guidance.

2.3. Total RNA isolation

The samples, which were obtained surgically, were homogenized. Afterwards total RNA was extracted from endometrial specimens using TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. RNA extracts were treated with DNase I to eliminate DNA (RNaesy Mini Kit, Qiagen, Valencia, USA). Isolated RNA was checked with the use of a spectrophotometer GeneQuant II RNA/DNA calculator (Pharmacia Biotech, Cambridge, UK). Next, quality analysis was performed using 1% agar electrophoresis stained with ethidium bromide. Only the positive outcome of both analyses was considered to be a qualifying result for further investigation *via* oligonucleotide microarray HG-U133A (Affymetrix Inc., CA, USA).

2.4. Oligonucleotide microarray HG-U133A

The first step of the microarray HG-U133A procedure was cDNA synthesis using SuperScript Choice System (Invitrogen Technologies, CA, USA). Afterwards the cDNA was purified with Phase Lock Gel Light (Eppendorf, Germany). Biotinylated cRNA was obtained with the use of a BioArray High Yield RNA Transcript Labeling Kit (Enzo Life Science, New York, USA). The cRNA was purified with an RNeasy Mini Kit (Qiagen GmbH, Germany) afterwards both quantity and quality were estimated. A Sample Cleanup Module (Qiagen GmbH, Germany) was used for the fragmentation of the cRNA and a hybridization solution using a GenChip® Expression 3′-Amplification Reagents Hybridization Control Kit according to the Gene Expression Analysis Technical Manual (Affymetrix Inc., CA, USA) was prepared. The hybridization products were stained with streptavidin–phycoerythrin. Fluorescence intensity signals were analyzed with GeneArray Scanner G2500A (Agilent Technologies, CA, USA). All of the aforementioned procedures were made according to the producers protocols.

2.5. Statistical analysis

The obtained fluorescence signals were normalized with the RMA (Robust Multichip Average) method. Statistical analysis of the results was performed using professional software – Gene Spring 13.0 (Agilent Technologies, CA, USA). The ANOVA with *post hoc* Tukey and Benjamini–Hochberg correction was applied. Hierarchical clusterization was carried out using the Ward method. The overrepresentation test with Bonferroni correction was done using the PANTHER classification system.

3. Results

3.1. Clinical characteristics and grouping

The selected study group showed clinical stage I (13 patients) and II (6 patients) according to the FIGO criteria. The tissue samples were grouped according to their pathomorphological grading: G1 – 5, G2 – 10, G3 – 4 (Table 1).

3.2. Tumor suppressor genes differential in endometrial cancer

Analysis was carried out for 2950 Id mRNA (the full list is placed in the Supplementary Material) related to tumor suppressor genes based on the NetAffx database. After normalization with the RMA method ($\log_2$) the results showed a normal distribution in the groups studied providing the confirmation needed to implement the ANOVA test with the Benjamini–Hochberg correction [10]. Hence, there were 163 statistically significant mRNAs ($p < 0.05$) in all cancer grades in comparison to the control (Table 2). The obtained results were implemented for clusterization by cancer grade using the Ward method (Fig. 1). The significant 163 mRNAs (Table 3) were divided, after hierarchical clusterization, into two groups. The G2 and G3 cancer specimens were clustered as one group. In turn, the low-grade (G1) cancer

Table 1
The clinical characteristics of patients enrolled in a molecular analysis.

	N	Age	BMI (kg/m^2)	Pregnancies				FIGO stage			Coexisting diseases	
		$x \pm$ SD	$x \pm$ SD	0	1	2	≥3	I	II	III	Arterial hypertension	Diabetes mellitus
Proliferative phase endometrium	5	46.3 ± 4.2	25 ± 2.5	0	1	3	1	–	–	–	2	1
Adenocarcinoma endometrioides, G1	5	55.3 ± 7.3	27.1 ± 4.6	0	1	3	1	5	0	0	2	2
Adenocarcinoma endometrioides, G2	10	56.4 ± 5.7	27 ± 6.4	2	5	2	1	6	4	0	7	3
Adenocarcinoma endometrioides, G3	4	54.3 ± 8.3	30.3 ± 4.9	0	1	2	1	2	2	0	3	1