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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

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

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Table 2
Number of statistically significant Id mRNA obtained from ANOVA with Benjamini–Hochberg correction and *post hoc* Tukey HSD in different cancer grades.

Number of differential Id mRNA for all cancer grades				
	$p < 0.05$	$p < 0.01$	$p < 0.005$	$p < 0.001$
Corrected *p*-value	163	55	43	21
ANOVA *post hoc* Tukey results				
Histological grade	Control (K)	G1	G2	G3
Control (K)	163	38	128	101
G1	125	163	74	55
G2	35	89	163	29
G3	62	108	134	163

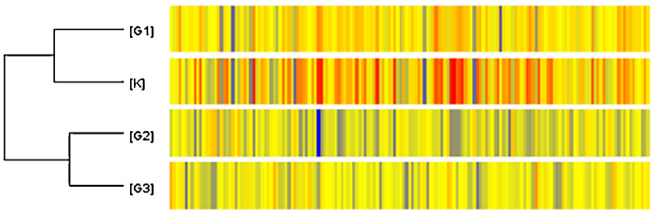

Fig. 1. Hierarchical clusterization with the Ward method for the ANOVA results.

were clustered as one group. In turn, the low-grade (G1) cancer was similar to the normal endometrium. Next, in order to determine specific changes of mRNA in various pathomorphological groups in comparison to the control *post hoc* Tukey HSD was employed (Table 2). Hence, after *post hoc* analysis there were 38 statistically significant mRNAs in grade 1 compared to the control, 128 in grade 2 and 101 in grade 3. The results obtained from the *post hoc* analysis were visualized on a Venn diagram, which showed 24 mRNAs common for all cancer grades (Fig. 2). Furthermore, the number of differential transcripts confirmed the low differentiation between the control and G1 grade where only 38 had been statistically significant, and 7 specific for grade 1 cancer. To determine significance in various biological processes the previously obtained 163 mRNAs (Table 3) were used to perform an overrepresentation test with the Bonferroni correction in the PANTHER classification system. The overrepresentation test with the Bonferroni correction resulted in the visualizing of biological processes and established the cell cycle as one of the most relevant for the TSGs selected. The genes were further checked in literature and internet databases (*e.g.* Genecards, Pubmed) to explore their biological significance. Afterwards the results obtained from the Panther analysis were correlated with the Venn diagram, where the biologically significant genes were localized in different groups on the diagram. Amongst the 24 mRNAs which differentiated in all grades of cancer compared to the control, the three following genes were biologically significant: *WT-1* (Wilms Tumor 1), *CYR61* (Cystein-Rich Angiogenic Inducer 61), *TSPYL5* (Testis-Specific Y-Encoded-Like Protein 5). Furthermore, specific genes were obtained from the 37 mRNA differentials for *G2-HNRNPA0* (Heterogeneous Nuclear Ribonucleoprotein A0) and *BCL2L2* (BCL2-Like 2), and from the 15 differentials in *G3 – BAK* (BCL2-Antagonist/Killer 1). The listed genes fold changes and regulation for every cancer grade and the control are visible in Table 4. The corrected *p*-value is only for the cancer grade in which the gene was a differential in comparison to the control.

4. Discussion

In spite of the different classification system there is a significant heterogeneity in biological, molecular and pathological features within endometrial cancer types. From the point of view of molecular biology research the traditional classification model of endometrial carcinogenesis is too general, and is under question [11,12]. For that reason it is necessary to create an integrated classification that can help develop treatment adequate for pathological grading and personalized adjuvant therapy [12,13]. Advances in diagnostic methods based on molecular biology including microarray analysis have contributed to a better understanding of endometrial carcinogenesis and suggest a heterogeneity conception concerning molecular subtypes of similar histology. The oncological aspect of systematic lymphadenectomy in the early stage of endometrial cancer remains a matter of debate [14]. Lymph node invasion is one of the most significant prognostic factors defining treatment and it correlates with histological grading. Hence, patients with high grade tumors can benefit the most from adjuvant therapy [14,15]. In cases of uncertain pathomorphological results the role of systemic lymphadenectomy becomes controversial. The possible risk of intra- and postoperative complications related to extensive surgery treatment often exceed the potential therapeutic benefits [14].

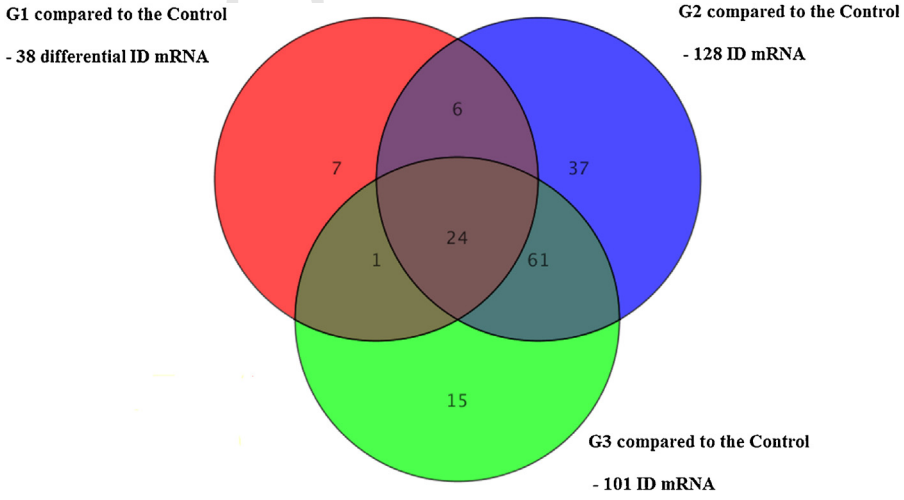

Fig. 2. Venn diagram – visualizing the number of differential Id mRNA in grades 1, 2, 3 in comparison to the control.

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Table 3
ANOVA results of 163 Id mRNA.

Gene symbol	*p* (Corr)	*p*	Cancer grade					
			G1		G2		G3	
			Fold change	Regulation	Fold change	Regulation	Fold change	Regulation
GAS2L1	0.0001	0.0000	−1.0853	Down	−1.4277	Down	1.8508	Up
PPARD	0.0343	0.0012	−1.0557	Down	1.0072	Up	1.4280	Up
STK11	0.0382	0.0015	1.5825	Up	1.0046	Up	1.0828	Up
EHD2	0.0028	0.0000	1.0040	Up	−2.9673	Down	−2.5949	Down
HIF1AN	0.0459	0.0023	−1.1143	Down	1.0265	Up	1.2361	Up
SPTBN1	0.0459	0.0022	1.4464	Up	1.8910	Up	−1.4980	Down
UBE2L3	0.0190	0.0003	−1.0986	Down	−1.6996	Down	−1.2231	Down
RPL10///SNORA70	0.0418	0.0018	1.1604	Up	−1.2037	Down	−1.4861	Down
SPARCL1	0.0425	0.0019	−3.4646	Down	−7.4605	Down	−3.3293	Down
RAB14	0.0253	0.0006	1.2348	Up	−1.0427	Down	1.2508	Up
CCND2	0.0252	0.0006	−2.2374	Down	−6.9895	Down	−5.3348	Down
TRIM28	0.0253	0.0006	1.4478	Up	−1.0480	Down	−1.3792	Down
HNRNPA0	0.0253	0.0007	1.0844	Up	1.8167	Up	−1.0096	Down
SMARCC1	0.0425	0.0019	1.5451	Up	1.9639	Up	1.1661	Up
SMARCC1	0.0097	0.0001	1.3151	Up	2.1734	Up	−1.2691	Down
BCLAF1	0.0498	0.0027	1.1470	Up	1.9790	Up	−1.4878	Down
TIMP3	0.0488	0.0026	−2.1478	Down	−5.4248	Down	−3.6040	Down
ENO1	0.0465	0.0024	−1.0875	Down	2.5031	Up	1.6786	Up
DAB2	0.0066	0.0001	−2.2552	Down	−4.2995	Down	−3.6329	Down
CYR61	0.0302	0.0009	−3.2508	Down	−4.7299	Down	−4.3093	Down
RBM5	0.0125	0.0002	−1.1229	Down	−1.8836	Down	−1.2524	Down
CLDN4///LOC100996451	0.0017	0.0000	−1.0203	Down	3.5832	Up	2.7749	Up
AIP	0.0237	0.0005	1.3193	Up	−1.0489	Down	−1.6320	Down
CAPG	0.0253	0.0007	1.1668	Up	2.5471	Up	1.5924	Up
ATMIN	0.0255	0.0007	−1.3633	Down	−1.8482	Down	−1.8320	Down
NR3C1	0.0302	0.0009	−3.0915	Down	−3.7246	Down	−1.8311	Down
NR3C1	0.0394	0.0015	1.0130	Up	−1.6285	Down	−1.5399	Down
ST14	0.0201	0.0004	1.3519	Up	2.3115	Up	2.4482	Up
SFRP1	0.0080	0.0001	−2.8718	Down	−14.7044	Down	−8.3432	Down
ARHGAP35	0.0395	0.0016	1.4200	Up	1.3799	Up	1.0577	Up
BLMH	0.0253	0.0006	1.0027	Up	−1.7170	Down	−1.7781	Down
DKK3	0.0017	0.0000	−1.7836	Down	−2.2646	Down	−2.2472	Down
N4BP2L2	0.0329	0.0011	−1.1576	Down	−2.2878	Down	−1.7520	Down
SFSWAP	0.0253	0.0006	1.0145	Up	−1.5591	Down	1.0511	Up
MAPKAPK3	0.0399	0.0016	1.6103	Up	1.8845	Up	1.6012	Up
MAPKAPK3	0.0257	0.0007	1.1014	Up	1.3686	Up	2.5602	Up
TRAF4	0.0063	0.0001	−1.0073	Down	1.6622	Up	1.9457	Up
MPP1	0.0459	0.0022	−1.1212	Down	−1.3703	Down	−1.6295	Down
CAV1	0.0063	0.0001	−2.0347	Down	−4.7245	Down	−4.6949	Down
TBP	0.0454	0.0021	1.0625	Up	−1.3881	Down	−1.3967	Down
RASSF2	0.0017	0.0000	−1.9066	Down	−4.7395	Down	−5.1592	Down
TUSC2	0.0247	0.0006	−1.0515	Down	1.4512	Up	1.3509	Up
LAD1	0.0102	0.0002	1.5846	Up	3.5726	Up	4.1584	Up
SOCS2	0.0201	0.0004	−1.5980	Down	−3.8018	Down	−3.9827	Down
SOCS2	0.0017	0.0000	−2.2817	Down	−5.5628	Down	−3.5247	Down
FEZ1	0.0066	0.0001	−1.8386	Down	−3.1640	Down	−1.8481	Down
DFNA5	0.0042	0.0000	−1.6485	Down	−3.8209	Down	−2.1531	Down
BAK1	0.0256	0.0007	1.0274	Up	−1.1030	Down	1.5753	Up
EMP3	0.0253	0.0006	−2.1109	Down	−3.1667	Down	−2.8096	Down
PCGF2	0.0410	0.0017	1.1852	Up	−1.3251	Down	−1.4323	Down
SFRP4	0.0028	0.0000	−2.7153	Down	−28.7876	Down	−11.9790	Down
SFRP4	0.0104	0.0002	−3.4245	Down	−12.7543	Down	−5.9449	Down
ULK2	0.0256	0.0007	−1.0346	Down	−1.8147	Down	−1.2714	Down
CDKN2C	0.0320	0.0010	−1.3113	Down	−1.8935	Down	−1.6593	Down
LOC101059993///RBM14	0.0042	0.0000	1.2489	Up	−1.2733	Down	−1.0752	Down
MNT	0.0136	0.0002	−1.3131	Down	−1.5018	Down	−1.1799	Down
GLIPR1	0.0459	0.0022	−2.4433	Down	−3.0658	Down	−3.3861	Down
GAS1	0.0498	0.0027	−1.9826	Down	−6.1602	Down	−3.1920	Down
GSTM1	0.0219	0.0005	−1.2329	Down	−3.1455	Down	−2.2746	Down
ME3	0.0405	0.0016	−1.0123	Down	−1.9794	Down	−1.3688	Down
CDH13	0.0193	0.0004	−1.2898	Down	−2.3091	Down	−2.3113	Down
PDS5B	0.0018	0.0000	−1.7139	Down	−2.3803	Down	−2.0633	Down
LEPREL2	0.0024	0.0000	1.1080	Up	−1.5732	Down	−2.1161	Down
SRPX	0.0085	0.0001	−2.5956	Down	−5.2222	Down	−5.3777	Down
AMPH	0.0359	0.0013	1.2494	Up	−1.7568	Down	−1.3068	Down
RECK	0.0399	0.0016	−1.2106	Down	−2.3629	Down	−2.1427	Down
RUNX1T1	0.0094	0.0001	−3.0291	Down	−6.5989	Down	−3.5205	Down
XPA	0.0343	0.0011	1.0029	Up	−1.4714	Down	−1.4859	Down
CYP27B1	0.0100	0.0001	1.5162	Up	1.0043	Up	1.2369	Up
SETBP1	0.0359	0.0013	−1.6968	Down	−2.8402	Down	−2.5749	Down
WT1	0.0010	0.0000	−3.4373	Down	−12.2219	Down	−7.4014	Down
CSNK2A1	0.0418	0.0018	1.2611	Up	1.4593	Up	−1.4968	Down
TPM1	0.0459	0.0022	−1.5189	Down	−2.1785	Down	−2.2496	Down

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Table 3 (*Continued*)

Gene symbol	*p* (Corr)	*p*	Cancer grade					
			G1		G2		G3	
			Fold change	Regulation	Fold change	Regulation	Fold change	Regulation
TCL1B	0.0410	0.0017	−1.1333	Down	1.1559	Up	1.0691	Up
HOXD3	0.0281	0.0008	1.0154	Up	−2.0580	Down	−1.5709	Down
EPB41L3	0.0425	0.0019	−1.3212	Down	−1.6738	Down	−1.5264	Down
ALDH1A2	0.0253	0.0006	−3.9054	Down	−14.9747	Down	−8.0961	Down
CC2D1A	0.0359	0.0013	1.3473	Up	1.4661	Up	1.1988	Up
DDR1///MIR4640	0.0425	0.0019	1.1001	Up	2.0673	Up	1.6596	Up
HOXD10	0.0219	0.0005	−1.3018	Down	−2.2039	Down	−2.6884	Down
SEPT9	0.0265	0.0008	1.5278	Up	1.6378	Up	−1.3732	Down
NCOR2	0.0359	0.0013	−1.0512	Down	−1.6966	Down	−1.2334	Down
SORBS3	0.0457	0.0021	1.2219	Up	−1.6809	Down	−2.2659	Down
TMEM8B	0.0465	0.0023	−1.1752	Down	−1.7082	Down	−1.5592	Down
PDS5B	0.0055	0.0000	−2.3291	Down	−2.6685	Down	−3.2705	Down
SMAD1	0.0459	0.0022	−1.2508	Down	−1.6311	Down	−1.2568	Down
LSR	0.0085	0.0001	1.4964	Up	3.1223	Up	3.1640	Up
MAPK1	0.0320	0.0010	1.1977	Up	1.0998	Up	−1.6610	Down
HIST3H3	0.0467	0.0024	−1.0504	Down	1.2162	Up	1.0689	Up
UBE2I	0.0244	0.0005	−1.0594	Down	−2.1772	Down	−1.6846	Down
THY1	0.0253	0.0007	−1.4792	Down	−5.9231	Down	−5.3205	Down
THY1	0.0265	0.0008	−1.9117	Down	−4.2577	Down	−3.6906	Down
PEG3	0.0051	0.0000	−3.0585	Down	−8.4380	Down	−4.3281	Down
PEG3	0.0463	0.0023	1.0800	Up	−1.4894	Down	−1.4394	Down
LOC100506403///RUNX1	0.0107	0.0002	1.1531	Up	2.1241	Up	2.2991	Up
IGF1	0.0063	0.0001	−4.8384	Down	−13.5357	Down	−5.0193	Down
IGF1	0.0055	0.0000	−6.1852	Down	−17.9723	Down	−10.0009	Down
IGF1	0.0399	0.0016	−2.1547	Down	−7.6552	Down	−5.9878	Down
NDN	0.0001	0.0000	−2.1349	Down	−8.6939	Down	−5.2276	Down
PTCH1	0.0189	0.0003	−3.5315	Down	−9.3953	Down	−7.0615	Down
PTCH1	0.0410	0.0017	−1.3837	Down	−2.6578	Down	−2.4005	Down
BIN1	0.0498	0.0028	1.1151	Up	−1.8189	Down	−1.8775	Down
ING1	0.0371	0.0014	1.0678	Up	1.2184	Up	−1.2565	Down
SPINT2	0.0418	0.0018	1.3047	Up	3.2981	Up	2.0206	Up
CYR61	0.0498	0.0027	−2.8421	Down	−6.3101	Down	−9.8954	Down
MEG3	0.0127	0.0002	−1.4070	Down	−3.4134	Down	−4.0857	Down
ERBB2	0.0247	0.0006	1.2499	Up	1.5968	Up	−1.0026	Down
IGF1	0.0201	0.0004	−2.1742	Down	−5.6270	Down	−4.7319	Down
MAST2	0.0339	0.0011	−1.3734	Down	−1.9415	Down	−1.7349	Down
LOC100506403///RUNX1	0.0498	0.0027	1.0338	Up	1.2804	Up	1.2518	Up
NR3C1	0.0253	0.0007	−2.3624	Down	−3.4800	Down	−2.1420	Down
CAV1	0.0201	0.0004	−2.6055	Down	−6.6337	Down	−4.2919	Down
CEBPB	0.0459	0.0022	−2.4179	Down	1.1160	Up	1.3332	Up
AKT3	0.0465	0.0024	−1.8695	Down	−3.5562	Down	−2.6249	Down
AKT3	0.0425	0.0019	−1.8879	Down	−2.4601	Down	−2.1260	Down
LOC100996668///ZEB1	0.0056	0.0000	−3.1107	Down	−7.4962	Down	−3.9029	Down
LYPD1	0.0352	0.0012	1.1722	Up	2.3137	Up	1.2143	Up
ANGPTL2	0.0201	0.0004	−1.6670	Down	−3.6235	Down	−3.3205	Down
ANGPTL2	0.0236	0.0005	−1.9867	Down	−2.7761	Down	−2.8895	Down
MAST3	0.0451	0.0021	1.1732	Up	−1.3712	Down	−1.1166	Down
STARD13	0.0352	0.0012	−1.5135	Down	−2.3487	Down	−2.1870	Down
TSPYL5	0.0001	0.0000	−3.1652	Down	−8.0634	Down	−5.0079	Down
TMEM158	0.0080	0.0001	−1.4235	Down	−5.8072	Down	−3.8469	Down
FLNA	0.0320	0.0010	−1.5008	Down	−3.6983	Down	−3.7506	Down
CD47	0.0201	0.0004	−1.2719	Down	1.9806	Up	3.6297	Up
THY1	0.0103	0.0002	−2.4953	Down	−5.8771	Down	−3.1659	Down
TRIM3	0.0086	0.0001	1.1124	Up	−1.5205	Down	−1.3751	Down
ZNF23	0.0371	0.0014	−1.3441	Down	−1.6897	Down	−1.0004	Down
PTPRD	0.0009	0.0000	−2.3433	Down	−3.1700	Down	−3.0832	Down
DKK3	0.0006	0.0000	−2.7106	Down	−5.2200	Down	−3.7479	Down
BIN1	0.0425	0.0019	1.0055	Up	−2.0570	Down	−1.9907	Down
H2AFY	0.0463	0.0023	−1.1034	Down	−1.7038	Down	−2.3208	Down
RABEP1	0.0410	0.0017	−1.1017	Down	−1.2298	Down	−1.8670	Down
MIR22///MIR22HG	0.0100	0.0001	−1.5565	Down	−1.9496	Down	1.0595	Up
MTUS2	0.0311	0.0010	1.0839	Up	−1.3566	Down	−1.3275	Down
BCL2L2	0.0330	0.0011	1.1998	Up	2.0449	Up	1.0657	Up
GSTM1	0.0256	0.0007	−1.0756	Down	−2.3902	Down	−1.8403	Down
STXBP5L	0.0498	0.0027	1.3667	Up	1.0299	Up	1.0552	Up
FANCA	0.0459	0.0022	−1.0158	Down	1.3347	Up	1.2175	Up
MAST2	0.0141	0.0002	−1.1912	Down	−2.3050	Down	−1.7790	Down
NR3C1	0.0257	0.0007	−1.3859	Down	−2.3406	Down	−1.5530	Down
MTAP	0.0371	0.0014	1.0578	Up	1.4728	Up	−1.0269	Down
PTPN12	0.0465	0.0024	1.0900	Up	1.3659	Up	−1.0656	Down
WT1	0.0063	0.0001	−1.8586	Down	−4.9163	Down	−4.5027	Down
KRT3	0.0498	0.0027	−1.0399	Down	1.4495	Up	1.0361	Up
SMAD3	0.0042	0.0000	−2.7032	Down	−3.4679	Down	−2.0351	Down
FAM188A	0.0100	0.0001	−1.2680	Down	−1.4306	Down	−1.2994	Down

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Table 3 (*Continued*)

Gene symbol	*p* (Corr)	*p*	Cancer grade					
			G1		G2		G3	
			Fold change	Regulation	Fold change	Regulation	Fold change	Regulation
KANK2	0.0051	0.0000	−2.3077	Down	−4.7010	Down	−3.1530	Down
DNMT3A	0.0367	0.0013	1.4502	Up	1.5368	Up	1.0203	Up
RNF43	0.0378	0.0014	1.2411	Up	1.7423	Up	−1.1243	Down
SIRT7	0.0085	0.0001	1.0581	Up	1.0371	Up	2.0391	Up
INTS6	0.0378	0.0014	1.0227	Up	−1.2542	Down	1.5177	Up
SCUBE2	0.0048	0.0000	−1.5801	Down	−4.9742	Down	−3.2531	Down
FAT4	0.0359	0.0013	−1.0537	Down	−1.8365	Down	−1.5551	Down
WFDC1	0.0002	0.0000	−1.2901	Down	−4.2749	Down	−3.5646	Down
ANGPTL2	0.0467	0.0024	−1.5247	Down	−2.2611	Down	−2.9653	Down
DET1	0.0047	0.0000	1.0829	Up	−2.2352	Down	−1.7057	Down
MAGEL2	0.0086	0.0001	−1.5012	Down	−2.4649	Down	−2.5297	Down
ASB12///MTMR8	0.0487	0.0025	1.4589	Up	1.0455	Up	1.1282	Up
WWOX	0.0418	0.0018	1.3799	Up	−1.0232	Down	−1.0196	Down
N4BP1	0.0343	0.0012	−1.2968	Down	−2.0402	Down	1.0575	Up
EHD2	0.0019	0.0000	−1.1945	Down	−3.2548	Down	−2.2442	Down
SIRT3	0.0498	0.0027	1.0551	Up	−1.4306	Down	−1.0148	Down

Table 4
Statistically and biologically significant tumor suppressor genes related to the cell cycle.

Gene symbol	Histological grade								
	G1			G2			G3		
	FC	Regulation	*p*	FC	Regulation	*p*	FC	Regulation	*p*
CYR61	−3.3	Down	0.030	−4.7	Down	0.030	−4.3	Down	0.030
WT-1	−3.4	Down	0.001	12.2	Down	0.001	−7.4	Down	0.001
TSPYL5	3.2	Down	0.0001	8.0	Down	0.000	5.0	Down	0.000
HNRNPA0	1.09	Up	–	1.81	Up	0.025	−1.0	Down	–
BCL2L2	1.2	Up	–	2.05	Up	0.032	1.06	Up	–
BAK1	1.02	Up	–	−1.1	Down	–	1.57	Up	0.025
TP53	1.09	Up	–	1.03	Up	–	−1.73	Down	–

FC, fold change; *p*, corrected *p* value.

Numerous studies have provided information about the role of tumor suppressor genes in endometrial carcinogenesis. The knowledge of TSG expression in different pathomorphological cancer grades can be a valuable additional factor in treatment strategy. The *Integrated genomic characterization of endometrial cancer* further confirms the importance of both tumor suppressors and cell cycle related genes [16]. The obtained TSG expression results in different cancer grades had presented a specific pattern of genes for every grade compared to the control. In spite of the fact that gene expression does not fully show the amount of the gene product it provides an insight into cell metabolism and indicates ongoing changes. The PANTHER results from the 163 mRNAs allowed grade specific tumor suppressor genes related to the cell cycle to be distinguished. However, the analysis failed to present any specific genes in the well-differentiated cancer (G1) that met the criteria. This can be explained by the small number of molecular changes that occur in low grade cancer in comparison to the control. Furthermore, this state had been confirmed by clusterization (Fig. 1) of the obtained statistically significant genes. Hierarchical clusterization did not display distinct separation between the low grade endometrioid *adenocarcinoma* and normal endometrial samples. However, the majority of differential transcripts in grade 1 cancer (Fig. 2A) were expressed regardless of their pathomorphological grading. These changes can possibly be considered to be the most primary in cancer development and remain relevant in further progression. Amongst the 24 differentially expressed genes, three met the criteria *WT-1*, *CYR61* and *TSPYL5* (Table 4).

WT-1 was characterized as a potential therapy aim for various cancer types [17]. The overexpression of *WT-1* has been regarded as a negative prognostic factor in serous endometrial cancer [18]. Studies in non-estrogen related female malignancies have shown that the overexpression of *WT-1* has a significant impact on cancer progression and negatively contributes to the patient survival ratio [19]. However, in a study performed by Alvarez et al. on estrogen-dependent high grade endometrioid endometrial cancer WT-1 was down-regulated [20]. This was confirmed in the results presented, furthermore, Wilms Tumor-1 was down-regulated in all cancer grades when compared to the control with a further decrease in expression in high-grade cancer (Table 4). Such data suggest that WT-1 down-regulation in endometrioid endometrial cancer has a positive effect on patient prognosis across all cancer grades.

Another differentially expressed gene was *CYR61*. That acts as a tumor suppressor gene in non-small-cell lung cancer by increasing p53 expression [21]. Recent studies show that *TP53* is over-expressed in approximately 23% of endometrioid endometrial cancers [22]. It has been shown that approximately 25% of grade 3 ECC has *TP53* mutations that result in an increase in gene expression [16]. However, other studies suggest that in high-grade (G3) endometrioid endometrial cancer its expression is down-regulated [9,23]. There are two former studies about *CYR61* expression in endometrial cancer cell lines. Both suggest that *CYR61* expression is regulated in an estrogen-dependent manner and can be a valuable prognostic factor for patients with endometrial cancer. They also agreed on *CYR61* influence on cell proliferation. However, Chien et al. [24] proved the decreased expression of the gene in EEC. The over-expression of *CYR61* had an impact on cell proliferation, whereas a knockdown with RNAi inhibited cell growth. In contrast MacLaughlan et al. [25] presented

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contradictive results showing the over-expression of the gene in endometrial cancer samples [24,25]. There was no data for either *TP53* mutations in the sample and/or p53 expression and downstream signaling. The microarray results confirmed Chien's [24] theory proving the significant down-regulation of gene expression in all cancer grades. In addition *TP53* gene expression was not statistically significant in the results presented and only in high-grade cancer (G3) does *TP53* fold change suggest down-regulation of the gene.

Another distinguished tumor suppressor gene was *TSPYL5* which was down-regulated in endometrial cancer samples (Table 4). This gene has been described in breast cancer where it indirectly influences p53 expression leading to its ubiqitination and inhibiting the target gene expression [26]. Expression of the three genes has differed depending on the grading. However, the expression of two genes is correlated with p53 signaling and can be highly influenced by mutation in this gene.

The results presented enabled the differentiation of cancer grades 2 and 3 in comparison to grade 1 and the control group. There is a significant rise in the number of specific genes for grade 2 and 3. However, a significantly changed gene expression was visible only for *HNRNPA0* and *BCL2L2* (Table 4). Furthermore, according to Panther analysis their function as tumor suppressor genes is related to the cell cycle. The *HNRNPA0* function as a tumor suppressor gene is still not fully understood. It probably influences mRNA stability and is involved in controlling the cell cycle in the DNA-damage checkpoint [27,28]. In turn the *BCL2L2* pro-survival role is well established in terms of apoptosis and the cell cycle [29]. In cancer cells the protein has the ability to block p53 driven apoptosis [29,30]. Furthermore, members of the BCL family had a confirmed translocation which can lead to an increase in expression, which probably resulted in reduced apoptosis [16]. The expression of *BCL2L2* and *HNRNPA0* was statistically significant and over-expressed only in grade 2 cancer. The result is the further deregulation of the cell cycle. Amongst specific genes for the high grade cancer (G3) was *BAK1*, which is a proapoptotic gene activated by p53 and BCL2 family members stimulating cell death [31]. The significant over-expression of *BAK1* suggests that in G3 tumors the cell cycle and the whole tumor suppressor system has not fulfilled its role.

The characterized genes and their expression pattern in endometrial cancer patients can become important in deciding treatment strategy. The expression of *WT-1*, *CYR61* and *TSPYL5* can have an impact on the necessity of performing lymphadenectomy. Another feature is that the three genes are correlated with p53 signaling. Hence, if the genes are overexpressed, a wider surgical approach should be favored. All genes which are specific for their grade can influence the outcome of adjuvant therapy because of their role in the cell cycle and apoptosis. Thus molecular diagnostics should be performed prior to surgery to ensure the best possible therapy.

5. Conclusions

We can conclude that the *WT-1*, *CYR61* and *TSPYL5* gene expressions are important in endometrial cancerogenesis and patient prognosis. Their regulation has an influence on survival and can be of importance in therapy. The TSGs related to the cell cycle in G2 and G3 endometrial adenocarcinoma are mostly correlated to apoptosis. Changes in the expression of BAK and BCL2L2 suggest pro-survival signaling and inhibition of apoptosis leading to uncontrolled cancer cell proliferation. However, further study on the topic is needed. The identified TSGs can be considered as potential prognostic markers of endometrial *adenocarcinoma* in Polish women.

Conflict of interest

None declared.

Financial disclosure

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.advms.2016.04.001.

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