The significance of the altered expression of lysophosphatidic acid receptors, autotaxin and phospholipase A2 as the potential biomarkers in type 1 endometrial cancer biology

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Abstract. In order to study lysophosphatidic acid (LPA) signaling associated with type 1 endometrial carcinoma (EC), we evaluated the LPA receptors (LPARs), autotaxin (ATX) and phospholipase A2 (PLA2) expression in EC and normal endometrium with correlation to clinicopathological features. We investigated LPAR1, LPAR2, LPAR3, LPAR4, ATX and PLA2 expression at mRNA and protein levels using quantitative real-time PCR and western blot analyses in 37 ECs and 10 normal endometria. All the examined LPARs (except for LPAR3 protein), ATX and PLA2 were overexpressed in cancerous compared to healthy endometrium. The studied ECs showed the highest LPAR2 and LPAR1 expression. Statistically positive correlations were found between depth of myoinvasion and levels of LPAR1, LPAR2 and PLA2 transcripts and proteins. We also found positive correlations between LPAR1, LPAR2, LPAR4 and PLA2 expression with the International Federation of Gynecology and Obstetrics (FIGO) stage. The expression of LPAR1, LPAR2 and PLA2 was positively associated with the age of patients. Positive correlations were found between the expression of LPAR1 mRNA, LPAR2 mRNA and protein and LPAR3 mRNA and body mass index (BMI) of the examined patients. We found no association between the expression levels of the studied factors and diabetes or hypertension among the examined patients. Owing to the highest LPAR2 and LPAR1 expression in EC and positive correlations of these two receptors with the depth of myoinvasion and the FIGO stage, we believe that LPAR2 and LPAR1 show promise as predictors of the EC progression as well as the main receptors responsible for LPA action in the EC tissue.

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Introduction

Endometrial adenocarcinoma (EC) is a major cause of morbidity and mortality for women worldwide. According to epidemiological data it is the fourth most common malignancy among women in Poland. The mortality ratio resulting from this cancer led to the twelfth place in terms of the causes of cancer deaths in Poland (1). The exact molecular mechanism of the ethiopatology of EC is still a matter of continuous interest. However, it is known that the most important risk factors for the development of this kind of cancer are unopposed estrogen exposure, genetic mutations and obesity (2). Endometrial cancers have been assigned, based on histological and molecular pathology observations, as two major types (3). Most common type I estrogen-dependent adenocarcinoma with endometrioid morphology (EC1). Type II cancers include more aggressive histological variants such as clear-cell and serous carcinomas and uterine carcinosarcomas. The risk of EC1 is reported to be linked with unopposed estrogen exposure and action (4-6). The above-mentioned hormonal changes correlated with the increased estrogen receptor (ER) β transcript abundance leading to increased proliferation of endometrial cells with an increasing frequency of mutations in the cells (7).

The biological effect of lysophosphatidic acid (LPA) in the human uterus is mediated through four major, G protein-coupled transmembrane receptors: LPA receptor (LPAR)1-LPAR4 (8). In the human body, two general pathways of LPA production have been demonstrated. In each pathway, at least two major phospholipase activities are required: phospholipase A2 (PLA2) and phospholipase D [(PLD), also called autotaxin (ATX)] (9,10). There are some studies in the literature that LPA signaling may play a role in pathogenesis of both benign and malignant endometrial tumors. Billon-Denis *et al* (11) presented LPA influence on the growth of leiomyomas or fibroids. Treatment of leio-

myoma tumor-derived cell line with LPA-entailed DNA synthesis through ERK activation (11). The authors also proposed that LPA produced in leiomyomas *in vivo*, may be involved in tumor cell proliferation (11). There are also studies indicating that LPA promoted endometrial cancer invasion via the induction of matrix metalloproteinase-7 (MMP-7) (12,13). However, the number of studies on the significance of LPA-dependent signaling in endometrial tumor cells is still limited. Additionally, there are certain conflicting data on the usefulness of the LPAR status, ATX or PLA2 expressions as the independent prognostic factors in endometrial cancer patients as well as on the possibility of LPA-dependent targeted molecular therapy in endometrial cancer.

The aim of our study was to investigate LPARs, ATX and PLA2 expression in type 1 endometrial cancer and normal endometrium with correlation to clinicopathological features.

Materials and methods

Patients and samples. The study was approved by the Local Ethics Committee of the Faculty of Medical Sciences, University of Warmia and Masuria in Olsztyn.

Tissue samples were obtained from 37 postmenopausal women who underwent total abdominal hysterectomy because of EC. Standard histopathological parameters were determined by the pathologist. In each case, age, the presence of hypertension, obesity and type 2 diabetes were determined. The age of patients ranged from 46 to 82 years (mean, 64 years). Tumor stage, age range and body mass index (BMI) of endometrial cancer patients are presented in Table I. For the control samples, normal endometrium explants of middle-to-late proliferative phase of menstrual cycle were obtained during hysterectomies due to uterine leiomyomas from 10 premenopausal women (age range, 33-56 years). The explants for gene and protein expression analyses were frozen in liquid nitrogen and kept at -80˚C until molecular studies were performed.

Table I. Representative clinicopathological characteristics of 37 endometrial cancers.

Characteristics	No. of cases
Tumor stage and grade	
Stage IA grade 1	6
Stage IA grade 2	7
Stage IA grade 3	1
Stage IB grade 2	7
Stage IB grade 3	3
Stage II	10
Stage III	3
Age (years)	
≤60	15
>60	22
Body mass index (BMI)	
18-24.9	6
25-30	9
>30	22

Total RNA extraction and reverse transcription (RT). Total RNA was extracted from tissue explants using TRIzol according to the manufacturer's instructions. RNA samples were stored at -80˚C. Before use, RNA content and quality was evaluated by spectrophotometric measurement and agarose gel electrophoresis. One microgram of each sample of total RNA was reverse transcribed using a QuantiTect Reverse Transcription kit (#205311; Qiagen). The RT reaction was performed in a total reaction volume of 20 μl, following the manufacturer's instructions and products stored at -20˚C until real-time PCR amplification.

Quantitative real-time PCR. The quantification of mRNA for the studied genes was conducted by real-time PCR using specific primers for *LPAR1*, *LPAR2*, *LPAR3*, *LPAR4*, *ATX* and *PLA2*. The results of mRNA expression were normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, an internal control) mRNA expression and were expressed as arbitrary units. This housekeeping gene was chosen using NormFinder software, comparing three candidate genes: *GAPDH*, *β-actin* and *H2A.1*. The primers were designed using an online software package (http://bioinfo.ut.ee/primer3/). Primer sequences and the sizes of the amplified fragments of all transcripts are shown in Table II. Real-time PCR was performed with an ABI Prism 7900 (Applied Biosystems Life Technologies, Foster City, CA, USA) sequence detection system using Maxima® SYBR-Green/ROX qPCR Master Mix (#K0222; Fermentas, Thermo Scientific, USA). The PCR reactions were performed in 384-well plates. Each PCR reaction well (10 μl) contained 3 μl of RT product, 5 μM each of forward and reverse primers and 5 μl SYBR-Green PCR Master Mix. Real-time PCR was performed under the following conditions: 95˚C for 10 min, followed by 40 cycles of 94˚C for 15 sec and 60˚C for 60 sec. Subsequently, in each PCR reaction melting curves were obtained to ensure single product amplification. In order to exclude the possibility of genomic DNA contamination in the RNA samples, the reactions were also performed either with blank-only buffer samples or in the absence of the reverse transcriptase enzyme. The specificity of PCR products for all examined genes was confirmed by gel electrophoresis and sequencing. The efficiency range for the target and the internal control amplifications was between 95 and 100%. For relative quantification of mRNA expression levels, the previously reported real-time PCR algorithm was used (14).

Western blot analysis. For immunoblotting, protein fractions were obtained from the tissue samples and total protein from the cells. Briefly, luteal tissues were homogenized on ice in RIPA buffer containing 150 mM NaCl, 50 nM Tris Base, pH 7.2, 0.1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate and 5 mM EDTA in the presence of the protease inhibitor cocktail (#11697498001; Roche). Lysates were then sonicated and centrifuged at 10,000 x g for 15 min at 4˚C. The protein samples were stored at -70˚C for further analysis. The protein concentration was determined according to Bradford (15). Equal amounts (50 μg) of membrane fraction were dissolved in SDS gel-loading buffer (50 mM Tris-HCl,

Table II. Primers used for real-time PCR.

Gene	Primer sequence	Fragment size (bp)	GenBank accession no.
LPAR1	5'-GGCTATGTTCGCCAGAGGACTAT-3'		
	5'-TCCAGGAGTCCAGCAGATGATAA-3'	135	NM_001401.3
LPAR2	5'-GCTCTGTCGAGCCTGCTTGTCTTC-3'		
	5'-ACAGTCTTGACCAGGCTGAGCGTG-3'	149	NM_004720.5
LPAR3	5'-AAACTTTCCTTTGGCTCTGGAC-3'		
	5'-ATTCCAGCGAAGAAATCGGC-3'	458	NM_012152.2
LPAR4	5'-GGGTGACAGAAGATTCATTGACTTCC-3'		
	5'-GGCCAGGAAACGATCCACACTA-3'	415	NM_001278000.1
ATX	5'-CGTGAAGGCAAAGAGAACACG-3'		
	5'-AAAAGTGGCATCAAATACAGG-3'	776	NM_006209.4
PLA2	5'-ACATCTGCAAAAGCGCAAGG-3'		
	5'-CCTGCTGTCAGGGGTTGTAG-3'	374	NM_024420.2
GAPDH	5'-CTGCACCACCAACTGCTTAG-3'		
	5'-GGGCCATCCACAGTCTTCT-3'	120	NM_002046.5

LPA, lysophosphatidic acid; LPAR, LPA receptor; ATX, autotaxin; PLA2, phospholipase A2.

Table III. Comparison of mRNA and protein levels of LPARs, ATX and PLA2 between studied endometrial cancers (EC) and normal endometrium tissues (control) using qRT-PCR and western blotting studies.

			qRT-PCR study			Western blotting study		
	Group	Number	Mean	SD	P-value	Mean	SD	P-value
LPAR1	EC	37	0.031	0.05	0.0001	2.5	1.5	0.05
	Control	10	0.005	0.002		1.7	0.7	
LPAR2	EC	37	0.03	0.05	0.0008	4.1	1.6	0.002
	Control	10	0.006	0.003		2.7	1.1	
LPAR3	EC	37	0.002	0.001	0.008	2.1	1.1	ns
	Control	10	0.0009	0.0007		1.8	0.7	
LPAR4	EC	37	0.001	0.0009	<0.0001	0.8	0.5	0.004
	Control	10	0.00007	0.0008		0.32	0.38	
ATX	EC	37	0.005	0.004	0.0006	1.3	0.6	0.0002
	Control	10	0.0014	0.0012		0.5	0.2	
PLA2	EC	37	0.004	0.004	0.0008	1.1	0.4	0.009
	Control	10	0.0014	0.0021		0.6	0.3	

LPA, lysophosphatidic acid; LPAR, LPA receptor; ATX, autotaxin; PLA2, phospholipase A2.

pH 6.8, 4% SDS, 20% glycerol and 2% β-mercaptoethanol), heated to 95˚C for 5 min and separated by 12% SDS-PAGE. Separated proteins were electroblotted using a semidry transfer method onto polyvinylidene difluoride membranes (Immobilon-P Transfer Membrane, #IPVH00010; Millipore) in transfer buffer (0.3 mM Tris buffer, pH 10.4, 10% methanol, 25 mM Tris buffer, pH 10.4, 10% methanol, 25 mM Tris buffer, pH 9.4, 10% methanol, 40 mM glycine). After blocking in 5% non-fat dry milk in TBS-T buffer (Tris-buffered saline with 0.1% Tween-20) for 1.5 h at 25.6˚C, the membranes were incubated overnight with rabbit polyclonal anti-LPAR2, ATX and cPLA2 antibodies (concentration 1:100, #sc-25490, #sc-66813 and #sc-438, respectively; Santa Cruz Biotechnology), rabbit polyclonal anti-LPAR1 and LPAR3 antibodies (concentration 4 μg/ml or 1:200, #10005280 and #10004840, respectively; Cayman Chemicals), goat polyclonal anti-LPAR4 (concentration 1:100; Santa Cruz Biotechnology #sc-46021) and monoclonal anti-GAPDH antibody produced in the mouse (concentration 0.05 μg/ml, #G8795; Sigma) at 4˚C. Subsequently, the proteins were detected by incubating the membranes with an anti-rabbit IgG-alkaline phosphatase antibody produced in the goat (concentration 1:20,000 for LPAR1, LPAR2, LPAR3, ATX and PLA2, #A3687; Sigma), donkey anti-goat IgG-alkaline phosphatase antibody (concentration 1:20,000 for LPAR4, #A4187; Sigma) or anti-mouse IgG-alkaline phosphatase antibody produced in the goat (concentration for all antibodies, 1:20,000 for GAPDH, #A3562; Sigma) for 1.5 h at 25.6˚C. After washing again in

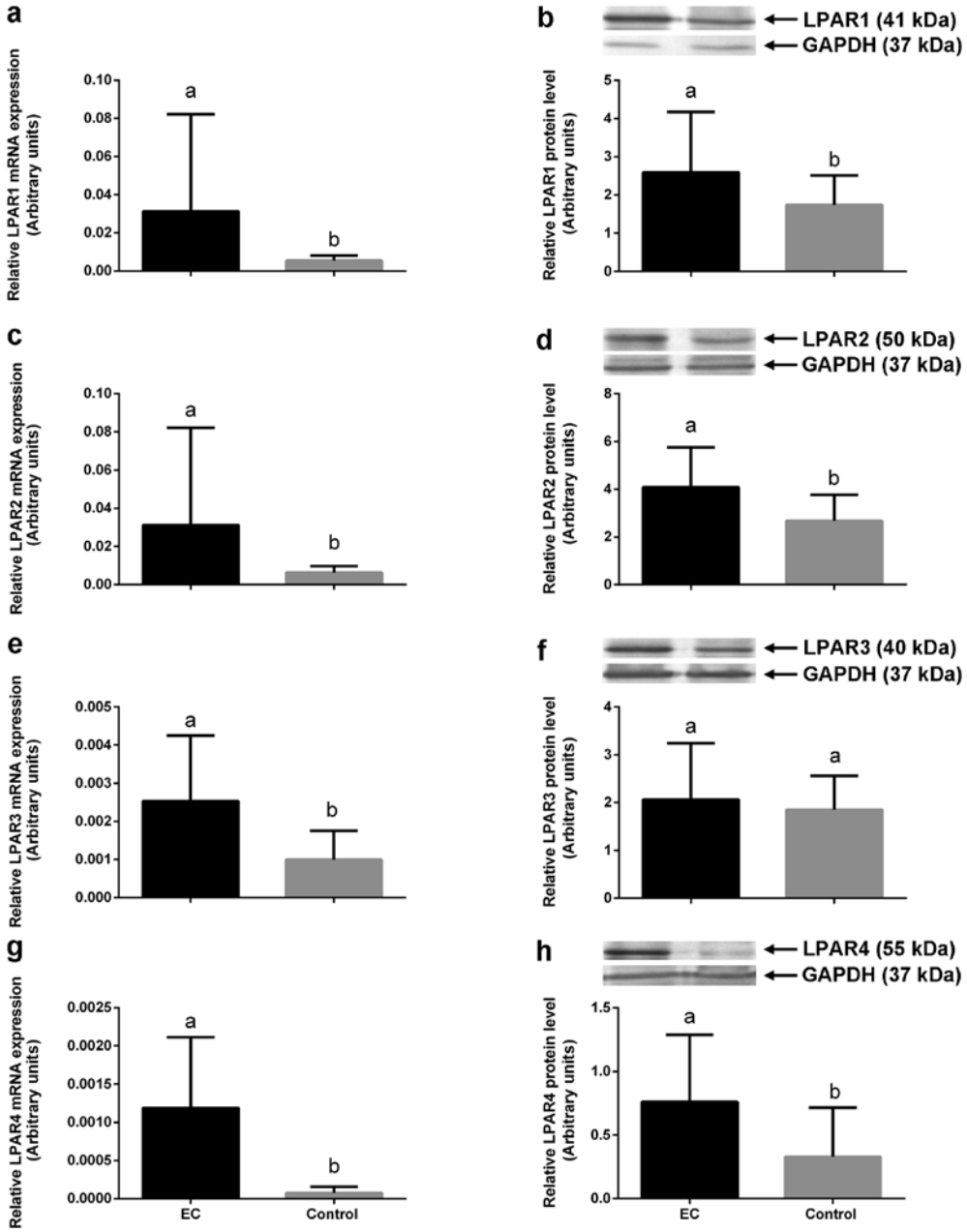

Figure 1. The expression of mRNAs (a, c, e and g) and proteins (b, d, f and h) for LPAR1, LPAR2, LPAR3 and LPAR4, respectively, in EC tissue (black bars) and normal endometrium (control, grey bars). All values are expressed as the mean ± SEM of LPAR1, LPAR2, LPAR3 and LPAR4 expression. Different letters indicate significant differences (P<0.05). LPAR, lysophosphatidic acid receptor; EC, endometrial cancer.

TBS-T buffer, the immune complexes were visualized using an alkaline phosphatase visualization procedure. The specific bands were quantified using Kodak 1D software (Eastman Kodak, Rochester, NY, USA). GAPDH was used as an internal control for protein loading.

Statistical analysis. Mean values ± standard deviation (SD) and median were calculated. The Student's t-test was used to compare normally distributed continuous variables and Mann-Whitney-U test for abnormal distribution. The Spearman's and Pearson's correlation coefficients were estimated. Linear regression analysis was also used. The analysis were performed using Statistica and GraphPad Prism software, accepting P<0.05 as significant.

Results

Expression profile of LPAR1, LPAR2, LPAR3, LPAR4, ATX and PLA2 in EC and normal endometrium. All the studied tumors as well as normal endometria expressed LPAR1, LPAR2, LPAR3, LPAR4, ATX and PLA2 mRNAs and protein levels. All the examined LPARs (except for LPAR3 protein) and enzymes responsible for LPA synthesis showed significantly higher mRNA and protein expression in cancerous than healthy endometrium (P<0.05). The cancer samples showed the highest LPAR2 and LPAR1 transcript and protein expression ranging from 0.001115 to 0.1907, mean 0.03125±SD 0.051 for LPAR2 mRNA and from 1.5 to 6.9, mean 4.1±SD 1.6 for LPAR2 protein in cancer tissue comparing to normal endo-

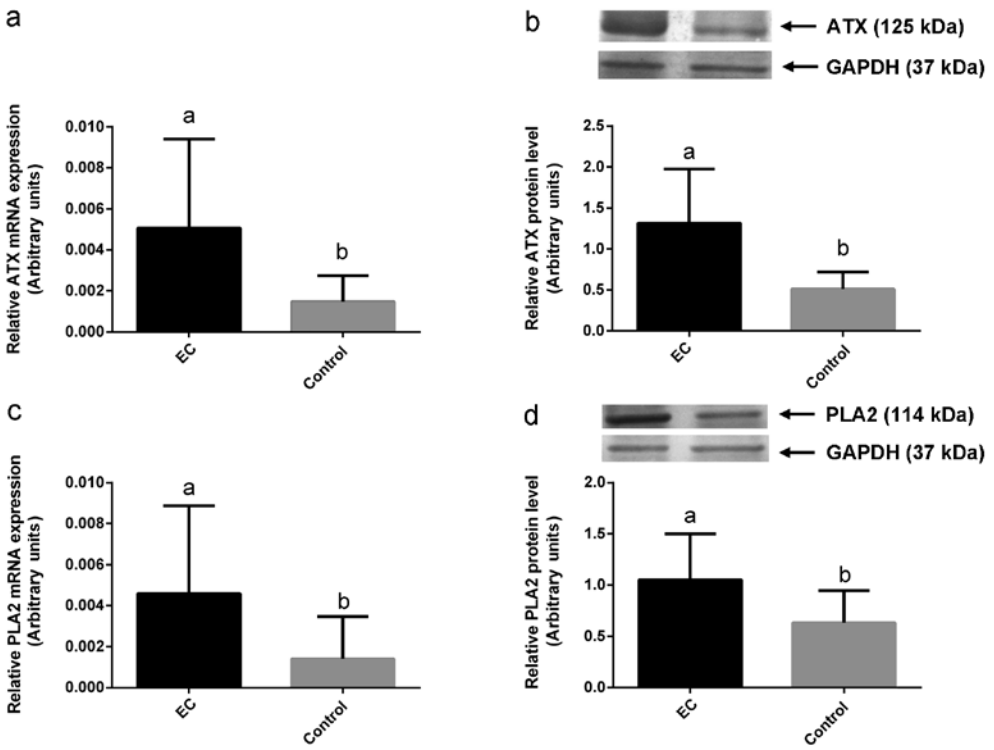

Figure 2. The expression of mRNAs (a and c) and proteins (b and d) for ATX and PLA2, respectively, in EC tissue (black bars) and normal endometrium (control, grey bars). All values are expressed as the mean ± SEM of LPAR1, LPAR2, LPAR3 and LPAR4 expression. Different letters indicate significant differences (P<0.05). ATX, autotaxin; PLA2, phospholipase A2; EC, endometrial cancer; LPAR, lysophosphatidic acid receptor.

metria ranging from 0.001 to 0.01, mean 0.0063±SD 0.0033 for LPAR2 mRNA and from 0.9 to 4.1, mean 2.6±SD 1.1 for LPAR2 protein (P<0.05, Table III and Fig. 1 c and d). LPAR1 mRNA expression in the cancerous tissue ranged from 0.001 to 0.19, mean 0.03±SD 0.05 and LPAR1 protein level from 1.1 to 6.9, mean 2.5±SD 1.5, whereas LPAR1 mRNA expression in normal endometria ranged from 0.0009 to 0.009, mean 0.005±SD 0.002 and LPAR1 protein level in healthy tissue ranged from 0.9 to 2.9, mean 1.7±SD 0.7 (P<0.05, Table III and Fig. 1 a and b). We also found significantly higher LPAR4, ATX and PLA2 transcript and protein expression in cancerous tissue (mean 0.0011±SD 0.009 for LPAR4 mRNA, mean 0.76±SD 0.52 for LPAR4 protein and mean 0.005± SD 0.004 for ATX mRNA, mean 1.3±SD 0.6 for ATX protein and mean 0.004±SD 0.004 for PLA2 mRNA, mean 1.05±SD 0.4 for PLA2 protein) comparing to normal endometria (mean 0.000074±SD 0.00008 for LPAR4 mRNA, mean 0.32±SD 0.38 for LPAR4 protein and mean 0.0014±SD 0.0012 for ATX mRNA, mean 0.5±SD 0.2 for ATX protein and mean 0.0014±SD 0.002 for PLA2 mRNA, mean 0.6±SD 0.3 for PLA2 protein) (P<0.05, Table III, Fig. 1 g and h and Fig. 2). We found significantly higher LPAR3 transcript expression in cancer tissue (mean 0.002±SD 0.001 for LPAR3 mRNA) comparing to normal endometria (mean 0.0009±SD 0.0007 for LPAR3 mRNA) (P<0.05, Table III and Fig. 1e). We did not find any difference in LPAR3 protein level between cancerous and normal tissues (P>0.05, Table III and Fig. 1f).

Correlations between LPARs, ATX and PLA2 expression with the selected clinical, pathological and metabolic features. Statistically positive correlations were found between depth of myoinvasion-pT category (where T1A-tumor limited to the endometrium or invades less than one half of the myometrium; T1B-tumor invades one half or more of the myometrium; T2-tumor invades stromal connective tissue of the cervix but does not extend beyond the uterus; and T3-tumor involves the uterine serosa, parametrium, vagina or adnexa) and levels of LPAR1, LPAR2 and PLA2 transcripts and proteins. In detail: LPAR1 was positively correlated with the depth of myoinvasion (P=0.00012, r=0.58 for mRNA and P=0.006, r=0.43 for protein, respectively), LPAR2 was positively correlated with the depth of myoinvasion (P=0.00012, r=0.58 for mRNA and P=0.00022, r=0.57 for protein, respectively), PLA2 was positively correlated with the depth of myoinvasion (P=0.0059, r=0.44 for mRNA and P=0.01, r=0.4 for protein, respectively). Additionally, we found positive correlations between LPAR3 and LPAR4 transcripts with the depth of myoinvasion (P=0.0003, r=0.56 for LPAR3 mRNA and P=0.0035, r=0.46 for LPAR4 mRNA, respectively). Interestingly, we also found positive correlations between LPAR1, LPAR2, LPAR4 and PLA2 mRNA and protein expression with the International Federation of Gynecology and Obstetrics (FIGO) stage. In detail: LPAR1 was positively correlated with FIGO stage (P=0.0022, r=0.48 for mRNA and P=0.000091, r=0.59 for protein, respectively), LPAR2 was positively correlated with FIGO stage (P=0.002, r=0.48 for mRNA and P=0.000015, r=0.64 for protein, respectively), LPAR4 was positively correlated with FIGO stage (P=0.001, r=0.51 for mRNA and P=0.017, r=0.38 for protein, respectively) and PLA2 was positively correlated with FIGO stage (P=0.0018, r=0.49 for mRNA and P=0.0008, r=0.6 for protein, respectively). Additionally, we found positive correla-

tions between LPAR3 mRNA and ATX protein with FIGO stage (P=0.0001, r=0.59 for LPAR3 mRNA and P=0.000001, r=0.7 for ATX protein, respectively).

We also found that the expression of LPAR1, LPAR2 and PLA2 at mRNA and protein level was positively associated with the age of patients (P=0.01, r=0.38 for LPAR1 mRNA; P=0.0056, r=0.44 for LPAR1 protein; P=0.019, r=0.38 for LPAR2 mRNA; P=0.0009, r=0.52 for LPAR2 protein; P=0.015, r=0.39 for PLA2 mRNA and P=0.005, r=0.44 for PLA2 protein, respectively). The expression of LPAR3 mRNA as well as LPAR4 and ATX protein levels were positively correlated with the age of the examined women (P=0.0038, r=0.46 for LPAR3 mRNA; P=0.027, r=0.36 for LPAR4 protein and P=0.003, r=0.47 for ATX protein, respectively). We found positive correlation between the expression of LPAR1 mRNA, LPAR2 mRNA and protein and LPAR3 mRNA with the BMI of the examined patients (P=0.047, r=0.32 for LPAR1 mRNA; P=0.047, r=0.32 for LPAR2 mRNA; P=0.03, r=0.36 for LPAR2 protein and P=0.02, r=0.38 for LPAR3 mRNA, respectively). We found no association between the expression levels of the studied factors and diabetes or hypertension amongst the examined patients (P>0.05).

Discussion

Cancer is a disease involving abnormal cell growth with the potential to invade or spread to other parts of the body. It is usually composed of cells of the impaired growth control mechanisms (16). Although there is a relatively high possibility for good prognosis for the early diagnosed cases of EC, there are still over 20% of deaths due to this carcinoma (17,18). This situation clearly reflects the failure of the available diagnostic tools in EC, especially in identifying its premalignant stages. Therefore there is still an urgent need for developing efficient prognostic markers and individual, targeted therapies for EC.

The results of many studies confirmed the important role of the LPA signaling system in the development of the reproductive organ related tumors, especially ovarian cancers. It was documented that LPA was produced by ovarian cancer cells and acted as the ovarian cancer activating factor (19-21). Moreover, LPA levels in the serum samples from ovarian cancer patients were much higher than in the serum samples from the group of healthy patients (22). Increased levels of LPA were also found in ascites of ovarian cancer patients and in the corresponding plasma samples (19,23-25). The *in vivo* performed studies using HEC1A, the EC cell line, demonstrated that the physiological level of LPA stimulated the invasion and proliferation of those cells (12,13). Moreover, Wang *et al* (13) reported LPA as a strong promoter of the urokinase plasminogen activator, with elevated levels correlating with tumor malignancy. Similarly, in our study all the examined enzymes responsible for LPA synthesis showed significantly higher mRNA and protein expression in cancerous than healthy endometrium. We found over 2 times higher ATX and PLA2 expression in cancerous tissue comparing to normal endometria. The data confirm the possibility of higher LPA synthesis and action in endometrial cancer compared to healthy uterus.

There are continuous efforts to establish whether different cellular effects of LPA on cell proliferation, motility and invasion in cancer cells depend on the activation of the certain type of LPARs. Of these, several studies documented the overexpression of LPAR2 and LPAR3 in ovarian cancer cell lines in comparison to normal ovarian epithelial cells (21,26,27). The elevated expression of LPAR2 and LPAR3 stimulated the migration and invasion of ovarian cancer cells (28). The data seem to be in agreement with the results of our study, where all the examined LPARs showed significantly higher mRNA and protein expression in cancer than healthy endometrium. The studied cancerous samples showed the highest LPAR2 and LPAR1 transcript and protein expression comparing to normal endometria. Moreover, the transcript and protein expression for LPAR4 was significantly higher in cancer tissue comparing to normal endometria. In case of LPAR3, only mRNA expression was significantly higher in cancer tissue comparing to normal endometria. Our data suggest that LPAR1 and especially LPAR2, with the highest expression in our study, may be mainly involved in LPA-induced proliferation and angiogenesis in the cancer tissue. Although, we did not examine that issues directly, there are data in the literature that LPAR2 was directly involved in the promotion of angiogenesis in ovarian tumors via the stimulation of vascular endothelial growth factor (VEGF) expression (26,29). Also, Fujita *et al* (30) documented the correlation between the LPAR2 and LPAR3 expression levels and the induction of VEGF expression in ovarian cancer cells. Moreover, the study of Yu *et al* (28) proved that the knockdown of LPAR2 and LPAR3 led to the suppression of the production of VEGF in ovarian cancer cells. Although, there is some information in the literature on the connection between LPA signaling and tumorigenesis in ovaries, LPA involvement in the ethiopathology of endometrial cancer is still not well examined. Most of already published studies were performed *in vivo* using the EC cell line, HEC1A. Hope *et al* (12) reported that among the 4 principle LPARs (LPAR1, LPAR2, LPAR3 and LPAR4), LPAR2 was predominantly expressed by HEC1A cells. This agrees with the data obtained in our study that endometrial cancer tissue show the highest LPAR2 transcript and protein expression compared to normal endometria. Wang *et al* (13) documented that the knockdown of LPAR2 caused the supression of the LPA-induced HEC1A invasion, but there were no significant changes in the level of migration of HEC1A cells (13). Besides, the knockdown of LPAR2 blocked LPA-induced activation of MMP-7 which usually plays an important regulatory role in cell surface proteolysis and is capable of binding to a variety of cell surface proteins, such as E-cadherin, β-integrin and tumor necrosis factor-α (13). In endometrial cancer, the overexpression of MMP-7 initiates the activation of MMP-2 which promotes cancer invasion (12). All of the above data point to LPAR1 and LPAR2 as the main receptors responsible for LPA action in the endometrial cancer tissue and at the same time the most promising predictors of the endometrial cancer progression.

To support the above-mentioned hypothesis, we found positive correlations between depth of myoinvasion and levels of LPAR1, LPAR2 and PLA2 transcripts and proteins. We also found positive correlations between LPAR3 and LPAR4 transcripts and the depth of myoinvasion. There were also positive correlations between LPAR1, LPAR2, LPAR4 and PLA2 mRNA and protein expression with the FIGO stage. Additionally, we found positive correlations between LPAR3

mRNA and ATX protein with FIGO stage. The expression of LPAR1, LPAR2 and PLA2 at mRNA and protein level and the expression of LPAR3 mRNA as well as LPAR4 and ATX protein levels were also positively associated with the age of patients. Moreover, we found positive correlation between the expression of LPAR1 and LPAR3 mRNA and LPAR2 mRNA and protein with the BMI of the examined patients. BMI is an unquestionable risk factor of endometrial cancer (31,32). Therefore, it is not surprising that LPA signaling connected with overexpression of the enzymes responsible for LPA synthesis and their receptors is associated with the excess of adipose tissue, as we have shown in the present study. Some other studies, focused on the increased BMI and treatment outcome in EC and demonstrated that elevated BMI was rather a favorable prognosticator (33-35). Although, there is often the association between BMI and hypertension and prognosis for tumor malignancy (36,37), in our study we found no association between the expression levels of the studied factors and diabetes or hypertension amongst the examined patients.

In summary, when we compared endometrial cancer versus non-cancerous endometrial tissue, we were able to show overexpression of all examined LPARs and enzymes responsible for LPA synthesis in cancer tissue. Especially, owing to the highest LPAR2 and LPAR1 transcript and protein expression in cancerous tissue and positive correlations of both these receptors with the depth of myoinvasion and the FIGO stage, LPAR2 and LPAR1 seem to be the most promising predictors of the endometrial cancer progression as well as the main receptors responsible for LPA action in the endometrial cancer tissue.

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