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Differential expression of lysophosphatidic acid (LPA) receptors in human ovarian carcinomas

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Summary

Objective: Aims of this study was to explore the expression of three lysophosphatidic acid (LPA) receptors, LPA1, LPA2, and LPA3, at mRNA and protein levels, and their possible mechanisms and biological significance in human ovarian carcinomas (OC). *Materials and Methods:* Seventy-seven cases of epithelial OC were confirmed by histological classification: 53 cases were serous OC, 16 were endometrioid OC, three were mucinous OC, and the remaining five were ovarian clear cell carcinoma and others. Additionally, there were 42 patients with well-differentiated to moderately differentiated OC, 35 with poorly differentiated OC, 17 cases of recurrent OC, 60 cases of non-recurrent, 17 cases of borderline tumors, and 28 cases were benign OC. Normal ovarian epithelium (n = 8, 2-3 mm-thick) from patients with uterine fibroids were chosen as the healthy controls. Reverse transcription-polymerase chain reaction (RT-PCR) detected LPA1-3 mRNA expression levels. Immunohistochemical (IHC) staining was used to identify the LPA receptors protein expression. *Results:* LPA1 mRNA and protein levels were significantly lower in epithelial OC than in normal ovarian epithelium, benign ovarian tumor, and borderline tumors, and the converse was true for LPA2 and LPA3. Furthermore, the mRNA and protein levels of LPA receptors showed no statistical significant differences among those OC patients in different histological tumor subtypes; similar results were also presented in those cases of different degrees of differentiation and LPA1 mRNA level was significantly lower in patients with recurrent OC compared to those with non-recurrent OC, yet no significant difference was observed regarding the protein expression of LPA1. In addition, the mRNA and protein expression levels of LPA2 and LPA3 were both apparently higher in patients with recurrent OC than those with non-recurrent OC. *Conclusion:* LPA and its receptors could have important roles in OC. Upregulated expression of LPA1 inhibits growth of OC, implying LPA1 could be a novel therapeutic target in OC. LPA2 and LPA3 are relevant to metastatic behavior and could be used as prognostic indicators in OC.

Key words: Epithelial ovarian carcinomas; Lysophosphatidic acid; Lysophosphatidic acid receptors; Reverse transcription-polymerase chain reaction; Immunohistochemical staining; Borderline tumors; Recurrent; Non-recurrent.

Introduction

Ovarian carcinoma (OC), is one of the most lethal tumors of the female reproductive organ and remains the fifth major cause of death related to gynecologic malignancy throughout the world [1, 2]. The fatality rate in OC ranks third after cervical and uterine cancer. However, mortality rates in epithelial OC, which is the most frequent type of OC, is at the highest rate among all types of gynecological tumors [3, 4]. OC affects 22,240 women each year and approximately 14,000 women died of this disease in 2013 according to Surveillance, Epidemiology and End Results data [5]. The prognosis in OC may easily be the poorest among gynecological cancer with an overall five-year survival rate of 45%, steeply dropping down to 20-25% for Stages III and IV due to lack of effective therapies for advance-stage OC [6, 7]. Although the etiology of OC is not well-understood, potential risk of being diagnosed with this disease is inversely proportional to the quantity of lifetime ovulations [8]. Thus, greater lifetime ovulations, low parity, nulliparity, nulligravity, infertility, early menarche, and late menopause appear to be leading risk factors for OC [9]. Furthermore, breast cancer susceptibility genes significantly enhance the lifetime risk of OC to 27%-44%, and the age and onset of OC is significantly earlier in women carrying breast cancer susceptibility gene mutations [9, 10]. Approximately, 75% of OC patients present with an advanced stage at diagnosis when the disease has already undergone metastasis. There is an urgent need for serum biomarkers that could accurately identify in early stage of OC to improve OC diagnosis and treatment outcomes [11-13].

Lysophosphatidic acid (LPA) is a water-soluble phospholipid signaling molecule. LPA has gained much attention in recent years for its wide-ranging effects in different target tissues [14, 15]. LPA is a multifunctional lipid mediator known for its ability to stimulate cell proliferation, cell migration and survival, smooth muscle cell contraction, platelet aggregation, and tumor cell invasion [16, 17]. Not surpris-

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ingly, LPA increased cell proliferation in various carcinoma cell lines, including OC and prostate cancer cells [18, 19]. LPA is found at relatively low concentrations in plasma but higher concentrations are seen in ascites fluid from OC patients [20]. LPA also plays an important role in metastatic capacity and reduced susceptibility to apoptosis in OC cell lines treated with cisplatin. Recent studies suggest LPA is produced by malignant ovarian epithelium and exerts its influence by interacting with G-protein-coupled receptors, including all six LPA receptors (LPA1-6) [21, 22]. Aberrant expressions of LPA receptors (LPA1, LPA2, and LPA3) have been found in human ovarian tumors. LPA1 is mainly expressed in normal ovarian tissues, whereas LPA2 and LPA3 shows high expression in OC [23, 24]. Therefore, the present study is aimed to investigate the expression status and levels of LPA 1-3 receptor mRNA and protein in epithelial OC, normal ovarian, benign, and borderline ovarian tumors. Further, the authors intend to identify new targets for treatment of OC within the pathways they investigated.

Materials and Methods

Ethics statement

The study was carried out with the approval of the Institutional Review Board of Zhongnan Hospital of Wuhan University. Tissue samples were collected after written informed consent.

Study subjects and tissue samples

Total resected specimens were collected from patients who received surgery at the Department of Oncology, Zhongnan Hospital of Wuhan University between February 2012 to May 2013. After postoperative pathological confirmation, a total of 77 cases of epithelial OC were collected: 53 samples were serous OC, 16 were endometrioid OC, three were mucinous OC, and the remaining five were ovarian clear cell carcinoma and others. Additionally, there were 42 patients with well-differentiated to moderately differentiated OC, 35 with poorly differentiated OC, 17 cases of recurrent OC, 60 cases of non-recurrent, and 17 cases of borderline tumors. Twenty-eight patient specimens with benign ovarian epithelial tumor were used as benign tumor control tissue and 17 cases of borderline tumors. As normal controls, ovarian epithelium (thickness: 2~3 mm) samples from eight patients with hysteromyoma were collected, and significant care was taken to exclude patients with other gynecologic tumors, pelvic inflammatory, vascular occlusive diseases, diabetes mellitus, hypertension and coronary disease, etc. All experimental subjects were confirmed with no pathogen reduction of platelets or other blood diseases, non-reproductive system cancers, stroke, paralysis, and other neurological diseases. The collected tissue samples were snap-frozen in liquid nitrogen stored at -80°C within two hours until further use.

RT-PCR for LPA(1-3) receptors

Tissue samples were immersed in Trizol reagent (100 mg/ml) and were then pulverized by mortar and pestle under ice-bath. Tissue samples were subsequently maintained in 1.5 ml EP tube for 5~10 minutes for the extraction of total RNA. The integrity of RNA was identified by 1.5% agarose gel electrophoresis. The absorbance (OD value) at 260 nm and 280 nm were read with ultraviolet (UV) spectrophotometer for measuring the purity and concentration of total RNA. The RNA samples with OD260/280 ratio (protein contamination) of 1.8 to 2.0 was used to determine a higher purity of those samples. The transcriptional levels of *LPA1, LPA2, LPA3* were detected by applying real-time detector. Complementary DNA (cDNA) was synthesized using RT-PCR amplification kit. The total volume of PCR reaction was 50 μL: SYBR Green Mix (32.5 μL), ddH_2O (14.5 μL), cDNA template (2 μL), as well as the forward and reverse primers (both 0.5 μL). Liquid paraffin (30 μL) was covered on the surface of PCR reaction system. The amplification was adopted under the following condition: predenaturation at 95°C for ten minutes, followed by denaturation at 95°C for 15 seconds, annealing at 60°C for 45 seconds; then underwent the stage of dissolution after 40 cycle: 95°C for 15 seconds, 60°C for one minute, 95°C for 15 seconds, and 60°C for 15 seconds. The relative expression levels of LPA receptors mRNA used the ABI Prism 7500 SDS Software for statistical analysis. Expression levels of LPA receptors in each sample was calculated and quantified by using the 2-ΔΔCt method ($\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{GAPDH}}$, $\Delta\Delta Ct = Ct_{\text{test samples}} - Ct_{\text{control samples}}$).The primers utilized in the PCR reaction synthesized are shown in Table 1.

Immunohistochemical staining

For detection of LPA1, LPA2, and LPA3 protein expressions, immunohistochemical staining was performed on conventional sections of paraffin-embedded tissue. After heat treatment at 60°C for ten minutes, the tissue sections were daparaffinized in xylene (2×10 minutes), hydrated in descending grades of ethanol (100%, 90%, 80%, and 70%, per five minutes), immersed in distilled water for five minutes and rinsed with phosphate-buffered saline (PBS) solution (3×5 minutes). After antigen denaturation using microwave oven, the sections were incubated with 3% H_2O_2 deionized water for 40 minutes at room temperature to quench endogenous peroxidase activity, and washed again with PBS solution (2×5 minutes). With the addition of normal goat serum (NGS) as blocking agent, the sections were incubated further at 37°C for 30 minutes, followed by the primary antibody staining at 4°C overnight. Further incubation was performed at room temperature for 60 minutes and then rinsed using PBS (3×5 minutes). After addition of secondary antibody, the sections were incubated at 37°C for 40 minutes in dark enclosure and washed with PBS (3×5 minutes). Negative control samples were incubated with PBS instead of primary antibody. After incubation at 37°C for 40 minutes with streptavidin-horseradish peroxidase (HRP), diaminobenzidine (DAB) color liquid was added and the sections were observed under the microscope to appropriately terminate the reaction. Finally, the sections were rinsed with tap water, counterstained using hematoxylin, dehydrated by gradient ethanol, and mounted with neutral gum. The protein expression of LAP was observed under the light microscope. Brownish yellow staining of cytoplasm were recorded positive. The negative control was treated with PBS. Ten high-power fields in each section were randomly selected (n = 100 per field), and the percentage of positive cells were calculated: ≤ 5%, 0 point; 6%-25%, 1 point; 26%-50%, 2 points; 51%-75%, 3 points; > 75%, 4 points. Based on staining intensity, the expression level of LAP was classified into: nonstaning (0 point); pale yellow (1 point); yellow (2 points); brown yellow (3 points), and the 0 point (-); 1~4 points (+); 5~8 points (++); 9~12 points (+++) were predefined. The "+", "++", '+++" were regarded as positive signals with observable increase in staining intensity with naked eye.

Statistics analysis

Data was analyzed using SPSS software 18.0 and was presented with mean ± standard deviation (SD). The comparisons of measurement data were carried out using *t*-test and variance analysis, and count data comparisons used the Chi-square test. The results

Table 1. — *Primers sequences for LPA1, LPA2, LPA3, and GAPDH.*

Genes		Primers sequences
LPA1	For	5'- TGGTGGTCATTGTGGTCATC -3'
	Rev	5'- CATAGTCCTCTGGCGAACATAG -3'
LPA2	For	5'- TTCCACCAGCCCATCTACTAC -3'
	Rev	5'- ACCAGCCCTCAAGTGAAAGTC -3'
LPA3	For	5'- GGGCGTTTGTGGTATGCTG -3'
	Rev	5'- GGTTCACGACGGAGTTGAG -3'
GAPDH	For	5'- CACCCACTCCTCCACCTTTG -3'
	Rev	5'- CCACCACCCTGTTGCTGTAG -3'

Table 2. — *Expression of LPA1, LPA2, and LPA3 mRNA in normal ovarian epithelium, benign ovarian tumor, borderline tumor, and epithelial OC.*

Type	Case	LPA1	LPA2	LPA3
Normal ovarian epithelium	8	8.26 ± 1.25*	3.06 ± 0.31*	3.72 ± 0.39*
Benign ovarian tumor	28	8.13 ± 1.68*	3.01 ± 1.29*	4.20 ± 1.16*
Borderline tumor	17	7.16 ± 1.12*	3.80 ± 1.29*	5.13 ± 0.91*
Epithelial OC	42	4.44 ± 1.28	6.73 ± 1.89	8.51 ± 1.78

* compared to normal ovarian epithelium, $p < 0.05$;
# compared to benign ovarian tumor, $p < 0.05$.

with $p < 0.05$ were considered statistically significant.

Results

Expression of LPA receptors in normal ovarian, benign ovarian tumor, and epithelial OC

As shown in Table 2, LPA1 receptor mRNA and protein expression profile in normal ovarian epithelium, benign ovarian tumor, borderline tumor, and epithelial OC was obtained by RT-PCR and IHC detection, respectively. The authors found significant differences in expression levels of LPA1, LPA2, and LPA3 mRNA and protein in normal ovarian epithelium, benign ovarian tumor, and epithelial OC. LPA1 mRNA and protein level were higher in normal ovarian epithelium (8.26 ± 1.25), benign ovarian tumor (8.13 ± 1.68), and borderline tumor (7.16 ± 1.12) compared to epithelial OC (4.44 ± 1.28), which was statistically significant (p = 0.00). Conversely, LPA2 and LPA3 protein expression levels in normal ovarian epithelium, benign ovarian tumor, and borderline tumor were significantly low when compared to epithelial OC (LPA2: 3.06 ± 0.31/3.01 ± 1.29/3.80 ± 1.29 *vs*. 6.73 ± 1.89, p = 0.00; LPA3: 3.72 ± 0.39/4.20 ± 1.16/5.13 ± 0.91 *vs*. 8.51 ± 1.78, p = 0.00, respectively) (Table 2). In the comparison of normal ovarian epithelium, benign ovarian tumor, and borderline tumor, LPA2 and LPA3 mRNA did not match the protein results, and no significantly differences was detected among the three groups (all $p > 0.05$).

Table 3. — *LPA 1-3 receptor mRNA expression of different tissue types of OC.*

Type	Case	LPA1	LPA2	LPA3
Serous ovarian carcinoma	53	4.34 ± 1.41	6.4 ± 1.74	8.37 ± 1.92
Mucinous ovarian carcinoma	3	5.41 ± 0.95	6.45 ± 1.30	7.58 ± 0.46
Endometrioid ovarian carcinoma	16	4.70 ± 0.82	7.75 ± 2.30	9.27 ± 1.51
Ovarian clear cell carcinoma and others	5	4.11 ± 0.93	7.01 ± 1.54	8.33 ± 0.98
p	-	0.386	0.092	0.256

Table 4. — *Expression of LPA receptors 1-3 mRNA and the differentiation and recurrence of epithelial OC.*

Type	Cases	LPA1	LPA2	LPA3
Degree of differentiation				
Well-to moderately differentiated	42	4.84 ± 1.09	6.53 ± 1.94	8.39 ± 1.79
Poorly differentiated	35	5.41 ± 0.95	6.45 ± 1.31	8.68 ± 1.80
P	-	0.282	0.420	0.797
Tumor recurrence				
Recurrent	17	3.21 ± 0.77	7.79 ± 1.40	9.629 ± 1.20
Non-recurrent	60	4.79 ± 1.18	6.43 ± 1.91	8.21 ± 1.81
p	-	0.000	0.042	0.048

LPA 1-3 receptor mRNA expression of different tissue types of OC

Table 3 shows the difference of LPA receptors mRNA expression in different histological types of epithelial OC based on the results of IHC analysis. As shown in Table 3, there was no significant difference of LPA 1-3 mRNA expression levels among serous cystadenocarcinoma of ovary, ovarian mucinous cystadenoma, endometrioid carcinomas, and ovarian clear cell carcinoma and others (all $p > 0.05$).

LPA receptor mRNA levels and the differentiation and recurrence of OC

Interestingly, no obvious differences in LPA 1-3 mRNA expression were found among patients with varying degrees of differentiation of epithelial OC (all $p > 0.05$), which are shown in Table 4. LPA1 mRNA expression levels in patients with recurrent OC were significantly lower than that in the non-recurrent OC (3.21 ± 0.77 *vs*. 4.79 ± 1.18), with statistical difference (p = 0.000). In contrast, LPA2 mRNA in the recurrent OC were higher compared to the non-recurrent OC 7.79 ± 1.40 *vs*. 6.43 ± 1.91, with no statistical difference (p = 0.042); similar results were also observed when comparing the mRNA levels of LPA3 between the recurrent and non-recurrent OC (9.629 ± 1.20 *vs*. 8.21 ± 1.81, p = 0.042).

Table 5. — *Expression of LPA1-3 protein in normal ovarian epithelium, borderline tumors, benign, and epithelial OC.*

Groups	Cases	LPA1		LPA2		LPA3	
		Low expression	High expression	Low expression	High expression	Low expression	High expression
Normal ovarian epithelium	6	1 (16.67)	5 (83.33)	5 (83.33)	1 (16.67)	5(83.33)	1 (16.67)
Benign ovarian cancer	28	9 (32.14)	19 (67.86)	23 (82.14)	5 (17.86)	22 (78.57)	6 (21.43)
Borderline tumors	17	7 (41.18)	10 (58.82)	12 (70.59)	5 (29.41)	12 (70.59)	5 (29.41)
Epithelial ovarian cancer	77	62 (80.52)	15 (19.48)	31 (40.26)	46 (59.74)	30 (38.96)	47 (61.04)
Total	128	79 (61.72)	49 (38.28)	71 (55.47)	57 (44.53)	69 (53.91)	59 (46.09)

Table 6. — *Expression of LPA1-3 mRNA in different histological stages of epithelial OC, and in the degree of differentiation and recurrence of epithelial OC (cases, %)*

Groups	Cases	LPA1		LPA2		LPA3	
		Low expression	High expression	Low expression	High expression	Low expression	High expression
Histological stages							
Serous ovarian carcinoma	53	44 (83.02)	9 (16.98)	20 (37.74)	33 (62.26)	17 (32.08)	36 (67.92)
Mucinous ovarian carcinoma	3	2 (66.67)	1 (33.33)	1 (33.33)	2 (66,67)	1 (33.33)	2 (66.67)
Endometrioid ovarian carcinoma	16	13 (81.25)	3 (18.75)	8 (50.00)	8 (50.00)	7 (43.75)	9 (56.25)
Ovarian clear cell carcinoma and others	5	3 (60.00)	2 (40.00)	2 (40.00)	3 (60.00)	2 (40.00)	3 (60.00)
Degree of differentiation							
Well- to moderately differentiated	42	34 (80.95)	8 (19.05)	22 (52.38)	20 (47.62)	15 (35.71)	27(64.29)
Poorly differentiated	35	28 (80.00)	7 (20.00)	11 (31.43)	24 (68.27)	13 (37.14)	22 (62.86)
Tumor recurrence							
Recurrent	17	16 (94.12)	1 (5.88)	3 (17.65)	14 (82.35)	3 (17.65)	14(82.35)
Non-recurrent	60	46 (76.67)	14 (23.33)	28 (46.67)	32 (53.33)	27 (45.00)	33 (55.00)

LPA receptor 1-3 expression in different ovarian tissue

To further explore the difference of LPA receptor expression in different epithelial ovarian tissues, LPA1-3 protein expressions were detected in normal ovarian epithelium, borderline tumors, benign and epithelial OC. The protein expression level of LPA1 in normal ovarian epithelium, borderline tumors, benign and epithelial OC were 83.33%, 67.86%, 58.82% and 19.48%, respectively, and expression levels in the epithelial OC was significantly higher than that in the previous three types (all $p < 0.05$). In addition, protein expression levels of LPA2 in the epithelial OC were apparently higher than that in the normal ovarian epithelium, borderline tumors, and benign OC (59.74% *vs*. 16.67% *vs*. 17.86% *vs*. 29.41%). Similar results were also detected with respect to the protein expression of LPA3 (61.04% *vs*. 29.41% *vs*. 21.43% *vs*. 16.67%), with statistical significance (all $p < 0.05$). No statistically difference was observed regarding the protein expression of LPA 1-3 in the normal ovarian epithelium, borderline tumors and benign OC (all $p > 0.05$) (Table 5).

LPA receptor expression correlated with different histological types, degree of differentiation and recurrence of epithelial OC

The protein expression levels of LPA receptors in serous, mucinous, endometrioid OC, and ovarian clear cell carcinoma and others are listed in Table 6. No statistically difference was observed between different histological types of ovarian carcinoma and LPA receptor expression (all $p > 0.05$). Furthermore, there was also no significant difference of LPA1 protein expression levels between the recurrent and non-recurrent OC (all $p > 0.05$). Yet LPA2 protein expression level was significant higher than that in the non-recurrent OC (82.35% *vs*. 53.33%), with statistical significance (all $p < 0.05$). There was also higher protein expression level of LPA3 in the recurrent OC when compared to that in the non-recurrent OC (82.35% *vs*. 55.00%), presented statistical analysis ($p < 0.05$).

Discussion

In the present study, the authors suspected that LPA1 had negative regulatory role in OC progression and that upregulated LPA1 expression might inhibit growth and survival of OC, implying that LPA1 may be an important therapeutic target for treatment of OC. The biological function mediated by LPA in various cancer via LPA receptors such as the LPA1-3 receptors is involved in cellular processes, for example, LPA binding to LPA receptors leads to downstream signaling leading to cell proliferation, differentiation, migration, and morphogenesis [12]. These LPA receptors themselves may have different biological mechanisms that are context and tissue dependent, since LPA receptor expression and tissue distribution is diverse [25].

With respect to those, the functions mediated through each LPA receptors vary, indicating that LPA receptors may have positive or negative effects on tumor development and progression [26]. LPA1 is generally regarded as negative regulator compared to LPA2 or LPA3 [27]; stabilized expression level of LPA1 can be regularly detected in normal and immortalized ovarian cells, whereas there were significantly differences when compared to epithelial OC, which were shown from the present results. Hence they support the observed inhibitory effects of LPA1 on proliferation and invasion through apoptosis and anoikis in OC [28]. LPA signaling pathway, via LPA1, inhibits cell motility and higher level of LPA1 was associated with decreased ability of cell proliferation, which consequently led to the acquisition of malignant potential of OC [29] and thereby blocking a series cascade of LPA1 may be a new target for treating ovarian cancer in future.

Importantly, the present results also demonstrate that elevated expression of LPA2 and LPA3 might be strongly associated with disease progression in OC. LPA-LPA receptors signaling promotes proliferation, migration and invasion, suggesting that increased LPA2 and LPA3 expression, may pose a significant risk in OC [30]. LPA2 and LPA3 overexpression had the increased tumor size and metastatic potential, correlating with the aggressiveness in ovarian carcinogenesis, which could be regarded as the potential biomarkers in predicting the progression of OC [31]. Mechanically, overexpression of LPA and LPA receptors activates Rho/Rho-associated kinase via the activation of G12/13 proteins, which function significantly in cytoskeletal remodeling, construction of actin stress fibers, as well as cell rounding, and stimulate the production of matrix metallopeptidase-9, which in-turn stimulates the invasion of OC cells [32]. Besides the above intracellular signaling regulations, LPA may also enrol the other protein-mediated signal transduction process, like the GTP binding proteins, that is not necessarily involved in cell signal transduction, activating mitogen-activated protein kinase kinase 1 (MEKK1), and is consequently responsible for various physiological and pathological events [33, 34]. Additionally, previous studies have shown that increased expression of LPA2 and LPA3 in OC tissues caused overproduction of vascular endothelial growth factor (VEGF), accelerating angiogenesis and providing microenvironment for tumor cell proliferation, metastasis, and invasion [35, 36].

Similarly, in different pathological grades of OC, the expressions of LPA2 and LPA3 in poorly differentiated stage were higher than those in the well to high differentiation group. Poorly differentiated stage play an important role in cell-cell adhesion, and exactly, adhesion and invasion of tumor cells is an important step in tumor metastasis, thereby hinting an aggressive process of epithelial OC [37]. In the present analysis of lipid composition of benign ovarian tissues and OC tumors, LPA receptors mRNA and protein levels were significantly lower in epithelial OC than in normal ovarian epithelium, benign ovarian tumor, and borderline tumors, and the converse was true for LPA2 and LPA3, indicating that LPA 2-3 can serve as prognostic markers for benign, borderline, and malignant tumors. Through LPA2 and LPA3 signaling, LPA can upregulate the expression of several angiogenic factors, such as interleukin-6, interleukin-8, growth regulated oncogene, and VEGF, thereby promoting the growth and metastasis of tumor as well [7]. LPA can also enhance the activity of matrix metalloproteinase in OC cells, to promote tumor invasion and metastasis [38]. This study also shows that the mRNA and protein expression levels of LPA2 and LPA3 were both apparently higher in patients with recurrent OC than those with non-recurrent OC, indicating that LPA2 and LPA3 may have potential role in predicting the development of OC. However, the mRNA and protein levels of LPA receptors showed no statistical significant differences among those OC patients in different histological tumor subtypes and in those cases of different degrees of differentiation. The present authors believe that with larger sample size it will be possible to clarify the issue. The present combined results indicate that LPA2 and LPA3 might be intimately associated with OC cell proliferation, migration, invasion, metastasis, and prognosis.

In summary, LPA and its receptors may play important roles in OC. LPA1 appears to play a negative role in the growth and metastasis of OC, therefore strategies to enhance LPA1 pathway may inhibit growth of OC and lead to novel treatments in OC. On the other hand, LPA2 and LPA3 appears to promote metastasis and therefore could be useful prognostic markers in OC.

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References

[1] Menon U.: "Ovarian cancer screening has no effect on disease-specific mortality". *Evid. Based Med.*, 2012, *17*, 47.
[2] Yung K.W., Yung T.T., Chung C.Y., Tong G.T., Liu Y., Henderson J., Welbeck D., Oseni S.: " Principles of cancer staging". *Asian. Pac. J. Surg. Oncol.*, 2015, *1*, 1
[3] Orfanelli T., Jeong J.M., Doulaveris G., Holcomb K., Witkin S.S.: "Involvement of autophagy in cervical, endometrial and ovarian cancer". *Int. J. Cancer*, 2014, *135*, 519.
[4] Liang Y., Chu P.H., Wang X.K.: "Health-Related Quality of Life of Chinese Earthquake Survivors: A Case Study of Five Hard-Hit Disaster Counties in Sichuan". *Soc. Indic. Res.*, 2014, *119*, 943.
[5] Marcus C.S., Maxwell G.L., Darcy K.M., Hamilton C.A., McGuire W.P.: "Current approaches and challenges in managing and monitoring treatment response in ovarian cancer". *J. Cancer*, 2014, *5*, 25.
[6] Zheng Y.F., Tan L.K., Tan B.H., Sterling H., Kane R.: "Principles of surgical oncology". *Asian. Pac. J. Surg. Oncol.*, 2015, *1*, 17.

[7] Liang Y., Lu W.Y., Wu W.: "Are social security policies for Chinese landless farmers really effective on health in the process of Chinese rapid urbanization? a study on the effect of social security policies for Chinese landless farmers on their health-related quality of life". *Int. J. Equity Health*, 2014, *13*, 1.
[8] Cahill G., Tran L., Baker R.A., Brown A., Huang Y.C., Ou C.W.: "Principles of radiation oncology". *Asian. Pac. J. Surg. Oncol.*, 2015, *1*, 27.
[9] Lin H.W., Tu Y.Y., Lin S.Y., Su W.J., Lin W.L., Lin W.Z., *et al.*: "Risk of ovarian cancer in women with pelvic inflammatory disease: a population-based study". *Lancet Oncol.*, 2011, *12*, 900.
[10] Liang Y., Cao R.X.: "Employment assistance policies of Chinese government play positive roles! The impact of post-earthquake employment assistance policies on the health-related quality of life of Chinese earthquake populations". *Soc. Indic. Res.*, 2015, *120*, 835.
[11] Dutta S., Wang F.Q., Wu H.S., Mukherjee T.J., Fishman D.A.: "The NF-κB pathway mediates lysophosphatidic acid (LPA)-induced VEGF signaling and cell invasion in epithelial ovarian cancer (EOC)". *Gynecol. Oncol.*, 2011, *123*, 129.
[12] Liang Y., Guo M.L.: "Utilization of Health Services and Health-Related Quality of Life Research of Rural-to-Urban Migrants in China: A Cross-Sectional Analysis". *Soc. Indic. Res.*, 2015, *120*, 277.
[13] Hunn J., Rodriguez G.C.: "Ovarian cancer: etiology, risk factors, and epidemiology". *Clin. Obstet. Gynecol.*, 2012, *55*, 3.
[14] Liang Y., Wang X.K.: "Developing a new perspective to study the health of survivors of Sichuan earthquakes in China: a study on the effect of post-earthquake rescue policies on survivors' health-related quality of life". *Health Res. Policy Syst.*, 2013, *11*, 1.
[15] Lee R., Yeung A.W., Hong S.E., Brose M.S., Michels D.L.: "Principles of medical oncology". *Asian. Pac. J. Surg. Oncol.*, 2015, *1*, 39.
[16] Houben A.J., Moolenaar W.H.: "Autotaxin and LPA receptor signaling in cancer". *Cancer Metastasis Rev.*, 2011, *30*, 557.
[17] Panupinthu N., Lee H.Y., Mills G.B.: "Lysophosphatidic acid production and action: critical new players in breast cancer initiation and progression". *Br. J. Cancer*, 2010, *102*, 941.
[18] Park S.Y., Jeong K.J., Panupinthu N.: "Lysophosphatidic acid augments human hepatocellular carcinoma cell invasion through LPA1 receptor and MMP-9 expression". *Oncogene*, 2011, *30*, 1351.
[19] Cahill T., Chen X.L., Lee J.W., Weiss M., Chang V.T., Cella D.: "Principles of radiofrequency ablation for cancer". *Asian. Pac. J. Surg. Oncol.*, 2015, *1*, 47.
[20] Snider A.J., Zhang Z., Xie Y., Meier K.E.: "Epidermal growth factor increases lysophosphatidic acid production in human ovarian cancer cells: roles for phospholipase D2 and receptor transactivation". *Am. J. Physiol. Cell. Physiol.*, 2010, *298*, 163.
[21] Ye X., Chun J.: "Lysophosphatidic acid (LPA) signaling in vertebrate reproduction". *Trends Endocrinol. Metab.*, 2010, *21*, 17.
[22] Dutta S., Wang F.Q., Wu H.S., Mukherjee T.J., Fishman D.A.: "The NF-κB pathway mediates lysophosphatidic acid (LPA)-induced VEGF signaling and cell invasion in epithelial ovarian cancer (EOC)". *Gynecol. Oncol.*, 2011, *123*, 129.
[23] Shin S.H., Kwon Y.W., Heo S.C., Jeong G.O., Kim B.R., Seo E.J., Kim J.H.: "Krüppel-like factor 4 mediates lysophosphatidic acid-stimulated migration and proliferation of PC3M prostate cancer cells". *Exp. Mol. Med.*, 2014, *46*, 104.
[24] Dobson P.R., Brown B.L., Beck D., Yang H., Zhou J., Voon Y.L.: "Management of surgical oncologic emergencies". *Asian. Pac. J. Surg. Oncol.*, 2015, *1*, 59.
[25] Hayashi M., Okabe K., Kato K., Okumura M., Fukui R., Fukushima N., Tsujiuchi T.: "Differential function of lysophosphatidic acid receptors in cell proliferation and migration of neuroblastoma cells". *Cancer Lett.*, 2012, *316*, 91.
[26] Tsujiuchi T., Araki M., Hirane M., Dong Y., Fukushima N.: "Lysophosphatidic acid receptors in cancer pathobiology". *Histol. Histopathol.*, 2014, *29*, 313.
[27] Murph M.M., Nguyen G.H., Radhakrishna H., Mills G.B.: "Sharpening the edges of understanding the structure/function of the LPA1 receptor: expression in cancer and mechanisms of regulation". *Biochim. Biophys. Acta*, 2008, *1781*, 547.
[28] Kato K., Yoshikawa K., Tanabe E., Kitayoshi M., Fukui R., Fukushima N., Tsujiuchi T.: "Opposite roles of LPA1 and LPA3 on cell motile and invasive activities of pancreatic cancer cells". *Tumour Biol.*, 2012, *33*, 1739.
[29] Hirane M., Araki M., Dong Y., Honoki K., Fukushima N., Tsujiuchi T.: "Inhibitory effects of LPA1 on cell motile activities stimulated by hydrogen peroxide and 2,3-dimethoxy-1,4-naphthoquinone in fibroblast 3T3 cells". *Biochem. Biophys. Res. Commun.*, 2013, *441*, 47.
[30] Ha J.H., Ward J.D., Varadarajalu L., Kim S.G., Dhanasekaran D.N.: "The gep proto-oncogene Gα12 mediates LPA-stimulated activation of CREB in ovarian cancer cells". *Cell. Signal.*, 2014, *26*, 122.
[31] Yu S., Murph M.M., Lu Y., Liu S., Hall H.S., Liu J., *et al.*: "Lysophosphatidic acid receptors determine tumorigenicity and aggressiveness of ovarian cancer cells". *J. Natl. Cancer Inst.*, 2008, *100*, 1630.
[32] Jeong K.J., Park S.Y., Cho K.H., Sohn J.S., Lee J., Kim Y.K., *et al.*: "The Rho/ROCK pathway for lysophosphatidic acid-induced proteolytic enzyme expression and ovarian cancer cell invasion". *Oncogene*, 2012, *31*, 4279.
[33] Alemayehu M., Dragan M., Pape C., Siddiqui I., Sacks D.B., Di Guglielmo G.M., *et al.*: "β-Arrestin2 regulates lysophosphatidic acid-induced human breast tumor cell migration and invasion via Rap1 and IQGAP1". *PLoS One*, 2013, *8*, e56174.
[34] Ghoneum M., Felo N., Nwaogu O.M., Fayanju I.Y., Jeffe J.A., Margenthaler D.B.: "Clinical Trials in Surgical Oncology". *Asian. Pac. J. Surg. Oncol.*, 2015, *1*,73.
[35] Fujita T., Miyamoto S., Onoyama I., Sonoda K., Mekada E., Nakano H.: "Expression of lysophosphatidic acid receptors and vascular endothelial growth factor mediating lysophosphatidic acid in the development of human ovarian cancer". *Cancer Lett.*, 2003, *192*, 161.
[36] Kitayoshi M., Kato K., Tanabe E., Yoshikawa K., Fukui R., Fukushima N., Tsujiuchi T.: "Enhancement of endothelial cell migration by constitutively active LPA(1)-expressing tumor cells". *Biochem. Biophys. Res. Commun.*, 2012, *422*, 339.
[37] August D.A., Kallogjeri D., Lewison G., Chen X.: "Nutrition support in surgical oncology". *Asian. Pac. J. Surg. Oncol.*, 2016, *2*, 153.
[38] Wang F.Q., Ariztia E.V., Boyd L.R., Horton F.R., Smicun Y., Hetherington J.A., *et al.*: "Lysophosphatidic acid (LPA) effects on endometrial carcinoma in vitro proliferation, invasion, and matrix metalloproteinase activity". *Gynecol. Oncol.*, 2010, *117*, 88.

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