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The lysophosphatidic acid (LPA) receptors their expression and significance in epithelial ovarian neoplasms

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Abstract

Objective. To investigate the lysophosphatidic acid (LPA) receptors expression situation and their biological significance in human ovarian cancer cell lines and in human epithelial ovarian neoplasms.

Methods. The reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting were employed to measure the expression levels of LPA_1, LPA_2 and LPA_3 mRNA, LPA_2 and LPA_3 protein expression in cultured human ovarian cancer cell lines (3AO, SKOV3 and OVCAR3) and in human epithelial ovarian neoplasms. The immunocytochemical method was used to detect LPA_2 and LPA_3 protein expression in cultured human ovarian cancer cell lines.

Results. RT-PCR revealed that all ovarian cancer cell lines expressed LPA_1, LPA_2 and LPA_3 mRNA. The positive rates (100%; 86.4%; 88.2%) of LPA_1 mRNA in normal ovarian tissue, benign tumor and ovarian cancer were no significant difference ($p>0.05$). The expression level of LPA_1 mRNA was significantly higher in normal ovarian tissue compared with that in benign tumor and in ovarian cancer tissue ($p<0.01$). LPA_1 expression level was no significant difference in both benign tumor and ovarian cancer tissue ($p>0.05$). LPA_2 mRNA-positive rates (92.6%) and expression level were significantly higher in ovarian cancer compared with that in benign tumor (31.8%) and in normal ovarian tissue (31.3%) ($p<0.01$); LPA_2 mRNA-positive rates and expression level were no significant difference in both benign tumor and normal ovarian tissue ($p>0.05$). LPA_3 mRNA-positive rates (92.6%) and expression level were significantly higher in ovarian cancer compared with that in benign tumor (31.8%) and in normal ovarian tissue (31.3%) ($p<0.01$), LPA_3 mRNA-positive rates and expression level were no significant difference in both benign tumor and normal ovarian tissue ($p>0.05$). LPA_1 mRNA expression level was significantly decreased compared with that of LPA_2 and LPA_3 in ovarian cancer ($p<0.01$); Western blotting clearly revealed that all ovarian cancer cell lines showed LPA_2 and LPA_3 protein. The positive rates and expression level of LPA_2 and LPA_3 protein were significantly increased in ovarian cancer (92.6%; 92.6%) compared with that in benign tumor (45.5%; 45.5%) and that in normal ovarian tissue (43.8%; 43.8%) ($p<0.01$); LPA_2 and LPA_3 protein-positive rates and expression level were no significant difference in both benign tumor and normal ovarian tissue ($p>0.05$). Correlation of clinicopathological parameters showed that LPA receptors mRNA and protein expression were associated with FIGO stage and histological grade, except pathologic types and age. The mRNA and protein expression of LPA_2 and LPA_3 in stages III and IV was significantly higher than that in stages I and II epithelial ovarian cancer ($p<0.05$). The mRNA and protein expression of pathologic grade G_3 was significantly higher compared with grade G_1 ($p<0.05$).

Conclusion. LPA_1, LPA_2 and LPA_3 mRNA and protein expressed widely in human epithelial ovarian neoplasms. LPA_2 and LPA_3 may be involved in the development and progression of human ovarian cancer. There was a significant correlation between LPA_2, LPA_3 and invasion and metastasis of epithelial ovarian cancer. LPA_2 and LPA_3 may be a prognostic indicator in patients with epithelial ovarian cancer.
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Keywords: Ovarian neoplasms; Lysophosphatidic acid (LPA); Receptor

Introduction

Lysophosphatidic acid (LPA) evokes various cellular responses in various cell types including cellular proliferation, prevention of apoptosis, cell migration, cytokine and chemokine secretion, platelet aggregation, smooth muscle contraction,

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and neurite retraction [1–3]. Previous reports have demonstrated that LPA stimulates proliferation, migration, matrix metalloproteinase (MMP) activation, and secretion of angiogenic factors in ovarian cancer cells, suggesting a positive role in the development of ovarian cancer [4–6].

Lysophosphatidic acid (LPA) induces various cellular responses by interacting with specific cell surface G-protein-coupled receptors of the endothelial differentiation gene (Edg) subfamily. LPA has been shown to interact with at least three receptors, LPA1/Edg-2, LPA2/Edg-4, and LPA3/Edg-7 [7,8].

Previous study indicated LPA2 and LPA3 higher level in tumor cells, especially in ovarian cancer cells [9]. Moreover, a high level of LPA has been detected in ascitic fluid from ovarian cancer patients [10,11]. These findings suggest that LPA and its receptors may be a mediator of tumour development and progression.

But there are few study about their expression and interaction role. Patterns of expression of these LPA receptors in tumors have not been adequately examined. In the present study we examined the expression patterns of LPA receptors in human ovarian cancer tissue at both the messenger RNA (mRNA) level and protein level, in order to better understand the potential role of LPA in the development of ovarian cancer.

Materials and methods

Cell lines and media

Cells were propagated in RPMI 1640 (Sigma) supplemented with 10% heat-inactivated FCS (Sigma) and 1000 U/ml penicillin/streptomycin. The ovarian cancer cell lines OVCAR-3, SKOV3 and 3AO were obtained from the Research Center of Hebei Medical University (Shijiazhuang).

Patients and surgical specimens

All the 106 patients in this study with tumor of low malignant potential and ovarian cancer had undergone surgery between July 2003 and December 2005 at the Department of Obstetrics and Gynecology, Fourth Affiliated Hospital, Hebei Medical University. Tissue specimens from ovarian tissue were snap-frozen in liquid nitrogen and then stored at −85°C until use. Histological types and differentiation of tumors were assessed by two pathologists according to the World Health Organization classification and suitable material from a representative area of each tumor was chosen for this study. There were 23 serous cystoadenocarcinomas, 23 endometroid adenocarcinomas and 22 mucinous cystoadenocarcinomas in 68 malignant ovarian tumors. Twenty-two patients had benign ovarian tumors, 4 serous cystoadenomas, 14 mucinous cystoadenomas, and 4 adenofibroma. Twenty-nine of malignant tumor were well differentiated, 25 moderately differentiated, and 14 poorly differentiated. FIGO stage was I and II (18); III and IV (50). The median age of the patient was 53 years old (range 18–69). Normal ovarian tissue specimens were also obtained at surgery for benign disease from 16 patients hysteromyoma. In this study, informed consent was obtained from all the patients.

Reverse transcription-polymerase chain reaction analysis

Total RNA was isolated using Trizol-Reagent (Invitrogen) according to the instruction from the manufacturer. Total RNA was extracted from each sample using the acid guanidine isothiocyanate/phenol/chloroform extraction method. 2 μg of total RNA from the samples was subjected to RT-PCR.

On the basis of the nucleotide sequences of LPA_1 cDNA, LPA_2 cDNA, LPA_3 cDNA (Aoke, Inc., China) 5′ ATC GGG ATA CCA TGA TGA GTC 3′ was used as the sense primer of LPA_1cDNA and 5′ TCC GTT CTA AAC CAC AGA GTG 3′ as the antisense primer of LPA_1 cDNA, 5′ GCT ACC GAG AGA CCA CGC TC 3′ was used as the sense primer of LPA_2 cDNA and 5′ CTG GGC AGA GGA TGT ATA GTG 3′ as the antisense primer of LPA_2 cDNA, 5′ ACA CCC ATG AAG CTA ATG AAG 3′ was used as the sense primer of LPA_3 cDNA and 5′ AGG CAT CCA GAG TTT AGG AAG 3′ as the antisense primer of LPA_3cDNA. Each reaction of mixture was subjected to 50 min of reverse transcription at 37°C and 35 RT-PCR amplification cycles of 5 min at 94°C, 15 s at 95°C, 60 s at 57°C and 30 s at 72°C, 7 min at 72°C for the amplification of LPA_1 cDNA and LPA_3 cDNA, 5 min at 94°C, 15 s at 95°C, and 60 s at 58°C for the amplification of LPA_2 cDNA. β-Actin DNA amplification was used as the internal PCR control; the sense primer was, 5′ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG3′ and the antisense primer was 5′CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC 3′.The reaction of the mixture was subjected to 35 amplification cycles of 5 min at 94°C, 15 s at 95°C, and 60 s at 58°C for the amplification of β-actin cDNA. The PCR products were electrophoresed on 1.5% agarose gels. LPA_1 mDNA, LPA_2 mDNA, LPA_3 mDNA were calculated as a ratio against the intensity of corresponding β-actin mDNA, analyzed by a Gel-analyzer (Sony, Tokyo, Japan).

Western blot analysis

Protein was extracted from ovarian cancer cell lines and 106 cancer and normal tissues. A measure of 50 μg of protein was electrophoresed in sodium dodecyl sulfate (SDS)-12% polyacrylamide gel for 3 h at 80 V. The separated proteins were electrophoretically transferred to immobilon (polyvinylidene difluoride). After blocking by 5% nonfat dried milk in Tris-buffered saline with 0.125% Tween 20 (TBS-T) for 1 h at room temperature, the membranes were blotted with an anti-LPA2, LPA3 antibody (provided by Junken Aoki) at a concentration of 0.1 μg/ml. After washing in TBS-T, the membranes were incubated with anti-rat immunoglobulin-horseradish peroxidase (1:1000 dilution) (Zhong shan, Biotechnology, Inc., China) for 1 h. Protein bands were detected by DAB Immuno Detects (Zhong shan, Biotechnology, Inc., China). The molecular weights of the protein standard were phosphorylase b (Mr 50,000; 40,000) LPA_2 protein and LPA_3 protein were calculated as a ratio against the intensity of corresponding β-actin protein, analyzed by a Gel-analyzer (Sony, Tokyo, Japan).

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (PC version 11.0). Each experiment was performed in duplicate or triplicate. All experiments were repeated at least three times. Statistical significance was estimated with Student's *t*-test for unpaired observations and analysis of variance (ANOVA). All data shown are means± SEM. $p<0.05$ was considered statistically significant.

Results

LPA receptors mRNA in ovarian cancer cell lines

Total RNAs from three ovarian cancer cell lines, SKOV3, 3AO and OVCAR3 were analyzed by RT-PCR for LPA_1, LPA_2

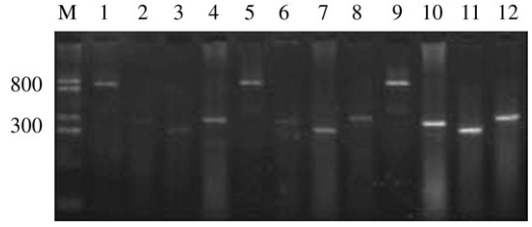

Fig. 1. Agarose gel electrophoresis of RT-PCR products of the LPA_1, LPA_2 and LPA_3 mRNA expression in ovarian cancer cell lines. Lanes 1–4 with 3AO; lanes 5–8 with OVCAR3; lanes 9–12 with SKOV3. Blots shown are representative of three experiments performed in duplicate, all with similar results.

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