Constitutive and Lysophosphatidic Acid (LPA)-induced LPA Production: Role of Phospholipase D and Phospholipase A₂

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ABSTRACT

Ascitic fluid and plasma from ovarian cancer patients, but not from patients with nongynecological tumors, contain elevated levels of the bioactive phospholipid lysophosphatidic acid (LPA). We show that ovarian cancer cells constitutively produce increased amounts of LPA as compared with normal ovarian epithelium, the precursor of ovarian epithelial cancer, or breast cancer cells. In addition, LPA, but not other growth factors, increases LPA production by the OVCA-3 ovarian cancer cell line but not by normal ovarian epithelium or breast cancer cell lines. We show that phospholipase D activity contributes to both constitutive and LPA-induced LPA production by ovarian cancer cells. Constitutive and LPA-induced LPA synthesis by ovarian cancer cells is differentially regulated with respect to the requirement of specific phospholipase A₂ (PLA₂) subgroups. Group IB (pancreatic) secretory PLA₂ plays a critical role in both constitutive and LPA-induced LPA formation, whereas group IIA (synovial) secretory PLA₂ contributes to LPA-induced LPA production only. Calcium-dependent and/or -independent cytosolic PLA₂s are required for constitutive LPA synthesis but do not play a role in LPA-induced LPA formation. LPA increases the proliferation of ovarian cancer cells, decreases sensitivity to cisplatin, the most commonly used drug in ovarian cancer, decreases apoptosis and anoikis, increases protease production, and increases production of neovascularization mediators. Thus, an understanding of the source and regulation of LPA production in ovarian cancer patients could identify novel targets for therapy.

INTRODUCTION

In the United States, ovarian cancer is the fifth most common female malignancy and is the leading cause of death from gynecological malignancies (1). In 1999, ~26,800 women will be newly diagnosed, and 14,500 will die from ovarian cancer (1). The majority of patients are diagnosed with advanced epithelial ovarian cancer with widespread metastatic disease. The dismal outcome for ovarian cancer results from an inability to detect the tumor at an early curable stage. As 90% of stage IA and 70% of stage II tumors can be cured by current management, ovarian cancer diagnosed at an early stage has a prognosis similar to breast cancer. The most likely way to identify ovarian cancer at an early, curable stage and to develop new, effective therapies for advanced ovarian cancers is to improve our understanding of the processes leading to the initiation and progression of this disease.

Ascitic fluid from ovarian cancer patients, but not from patients with other cancers or with benign diseases such as hepatic disease, contains elevated levels of the phospholipid LPA (2–5). LPA levels are also significantly elevated in plasma from >90% of patients with ovarian cancer regardless of stage (6). In contrast, LPA levels are not elevated in plasma of patients with breast cancer or leukemia or in healthy controls (6). LPA levels are also increased in patients with endometrial cancer and cervix cancer (6), multiple myeloma (7), and renal dialysis (8), all of which can be clinically distinguished from ovarian cancer. This suggests that LPA in plasma might provide a marker for diagnosis of ovarian cancer, establishing prognosis, or monitoring response to therapy. Because LPA levels are also elevated in the early stages of the disease (6), the plasma LPA assay offers the possibility of earlier diagnosis of ovarian cancer, resulting in improved prognosis.

LPA displays a broad spectrum of biological activities (9–12). Its principle effects are growth related, such as induction of cellular proliferation and suppression of apoptosis, or involve the cytoskeleton or adhesive proteins contributing to aggregation, adhesion, contraction, secretion, and chemotaxis. LPA stimulates the growth (4, 13), prevents apoptosis (14) and anoikis (not presented), decreases sensitivity to chemotherapeutic drugs (15), and increases invasiveness of ovarian cancer cells.
(12). These effects are associated with increased phosphorylation of focal adhesion kinase, increased tyrosine phosphorylation of cellular proteins, increased intracellular calcium concentration, and increased MAPK activity after treatment with LPA (13). In contrast, normal ovarian epithelial cells are resistant to the effects of LPA (16), suggesting that acquisition of LPA responsiveness is associated with transformation. LPA acts on G protein-coupled receptors encoded by the endothelial differentiation gene (Edg) subfamily (17). The LPA and sphingosine-1-phosphate receptor Edg1 (18–20) is expressed at high levels in normal and immortalized ovarian epithelial cells but at low levels in most ovarian cancer cell lines (12). The LPA receptor Edg2 (21) is expressed by both normal ovarian epithelial cells and ovarian cancer cell lines at varying levels (12, 22, 23). In contrast, the LPA receptors Edg4 (24) and Edg7 (25) are expressed at relatively high levels in ovarian cancer cell lines but only at very low levels in normal and immortalized ovarian epithelial cells (12, 22). Binding of LPA to its receptor(s) activates pertussis toxin-sensitive (G_i) and -insensitive (G_q and G_12/13) pathways (10, 11), leading to the expression of growth factor-regulated genes that contain serum response elements. LPA is a normal constituent of serum (present at concentrations ranging from 1 to 5 μM), where it is produced and released by activated platelets (26). LPA is also produced by growth factor-stimulated fibroblasts (27), cytokine-stimulated leukocytes (11), PMA-activated ovarian cancer cells (28), and possibly by other cell types. Little is known, however, about LPA production in vivo and why LPA levels are elevated in ovarian cancer patients.

LPA may be synthesized by cells either de novo from glucose through pathways of lipid metabolism in the endoplasmic reticulum or through liberation of precursor phospholipids and subsequent enzymatic conversions in membrane microvesicles (11, 29, 30). The latter pathway is considered the principal source of production of free and secreted LPA. PLD first converts phosphatidylcholine to PA. Two distinct isoforms of PLD have been identified (31, 32). PLD1, but not PLD2, is activated by GTP-binding proteins and protein kinase C. Both PLD1 and/or PLD2, whereas LPA-induced LPA production is primarily dependent on group IB (pancreatic) sPLA2 and on cPLA2 and/or iPLA2, whereas LPA-induced LPA production was dependent on both group IB (pancreatic) and group II A (synovial) sPLA2, but not cPLA2 or iPLA2.

### MATERIALS AND METHODS

#### Reagents.
LPA (oleoyl, 18:1), EGF, and PDGF were purchased from Sigma Chemical Co. (St. Louis, MO). Fatty acid-free BSA was obtained from Boehringer Mannheim (Indianapolis, IN). Manoalide, OOEPC and AACOCF3 were obtained from Calbiochem (San Diego, CA). [32P]Pi (8810 Ci/mmol) was purchased from DuPont NEN (Boston, MA).

#### Cell Lines and Media.
Cells were propagated in RPMI 1640 (Central Core Media Facility, University of Texas M.D. Anderson Cancer Center) supplemented with 10% heat-inactivated FCS (Sigma) and 1000 units/ml penicillin/streptomycin (Life Technologies, Inc., Grand Island, NY). The ovarian cancer cell lines OVCAR-3 and SK-OV-3 were obtained from the American Type Culture Collection (Rockville, MD). The ovarian cancer cell line HEY was kindly provided by Dr. Ron Buick (University of Toronto, Toronto, Ontario, Canada). A2780.6.3 is a subclone of the ovarian cancer cell line A2780 (kindly provided by Dr. Thomas Hamilton, Fox Chase Cancer Center, Philadelphia, PA) stably expressing Edg-2 (23). The breast cancer cell lines MCF7, MDA-MB-231, and MDA-MB-468 were kindly provided by Dr. Janet Price (University of Texas M. D. Anderson Cancer Center). Normal ovarian epithelial cells...
(NOE35) were obtained in-house, and immortalized ovarian epithelial cells (IOSE29, IOSE80) were kindly provided by Dr. Nellie Auersperg (University of British Columbia, Vancouver, British Columbia, Canada).

**In Vivo Labeling and Stimulation of Cells.** Cells (0.2–0.8 × 10⁶) were plated in 60-mm dishes in complete medium. After 2 days, at 80% confluency, the cells were starved by removal of complete medium and addition of serum-free medium. Twenty-four h later, the cells were washed with phosphate-free medium and incubated in phosphate-free medium for 1 h. The cells were again washed with phosphate-free medium and incubated with 0.1 mCi [³²P]Pi/ml in phosphate-free medium for 1 h. The labeling medium was removed, and the cells were washed with serum-free medium and either treated with inhibitor for 20 min or immediately stimulated with 25 µM LPA for 2 h. Preliminary time course experiments had shown that maximum LPA production and release occurred after 2 h of LPA stimulation; therefore, this time point was used throughout. LPA was added to the cells in a solution of 1% fatty acid-free BSA in PBS. We therefore routinely tested fatty acid-free BSA for the presence of trace amounts of LPA.

**Lipid Extraction and Analysis of Phospholipids by TLC.** In thrombin-activated platelets, 90% of newly generated LPA is released into the medium (26). Furthermore, preliminary experiments showed that LPA produced by ovarian cancer cells was not retained within the cells but released into the extracellular space. Therefore, cell supernatants were used as source for extraction of phospholipids. After stimulation, the cell supernatant was removed and cleared by centrifugation at 14,000 × g for 5 min. Acetic acid was added to the samples to a final concentration of 20 mM. The samples were then extracted with 1-butanol and centrifuged. The 1-butanol phase was removed, and the aqueous phase was again extracted. The 1-butanol phases were combined and washed twice with 1-butanol-saturated water. The extracted lipids contained in the 1-butanol phases were dried, dissolved in chloroform:methanol (1:1), and loaded onto TLC plates (precoated silica gel 60 plates; EM Separations Technology, Gibbstown, NJ). Phospholipids were separated by two-dimensional TLC with the first dimension system containing chloroform:methanol:ammonium hydroxide (13:7:1.1) and the subsequent buffer system containing chloroform:methanol:88% formic acid:water (11.5:6.1:0.2). Phospholipids were detected by autoradiography and identified by comigration with nonradioactive marker lipids. Quantitation of LPA-containing spots was performed by PhosphorImager. PhosphorImager units were normalized with respect to the total amount of [³²P]-labeled phospholipids, which minimizes variability in cell numbers or in [³²P] labeling. Each experiment was performed at least twice, and the repeat experiment(s) yielded similar results. In lipid extracts from SK-OV-3 cells, we routinely saw a second minor spot running slightly further than the major LPA spot in the second dimension, which may represent alkonyl-LPA and was included in the LPA analysis.

**Total RNA Preparation and Northern Blot Analysis.** Total cellular RNA was isolated from normal and immortalized ovarian epithelial cells and various ovarian cancer cell lines using a RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Equal amounts of total RNA were separated by electrophoresis on denaturing 1% agarose gels and transferred to Hybond N⁺ membranes (Amersham, Arlington Heights, IL). Edg-7 and 185 RNA probes were radiolabeled by random-prime labeling using the Redi-Prime labeling kit (Amersham). Membranes were incubated with radiolabeled probes in 50% formamide, 10× Denhardt’s solution, 0.1% SDS, 4× SSC, 10 mM EDTA, and 100 µg/ml salmon sperm single-strand DNA (Sigma) at 42°C for 18 h. The blots were washed at room temperature in 1× SSC, 0.1% SDS for 20 min three times and then at 50°C in 0.1× SSC, 0.1× SDS for 20 min three times prior to autoradiography at −80°C for 1–2 days or analysis with PhosphorImager. Quality and comparable loading of RNA were confirmed by rehybridization of the membranes with radiolabeled 18S RNA.

**RESULTS**

**Analysis of LPA Synthesis by Ovarian and Breast Cancer Cell Lines.** The bioactive phospholipid LPA is present at elevated levels in the ascites and plasma of patients with ovarian cancer (2–6) and has been shown to exhibit pleomorphic activities on ovarian cancer cells (12). Levels of LPA are higher in ascites (up to 80 µM) than in plasma (up to 10 µM) from ovarian cancer patients, suggesting that LPA is produced in the peritoneal cavity and then migrates to the peripheral circulation. Indeed, LPA levels averaged 4-fold higher in matched ascites as compared with plasma samples from ovarian cancer patients. In each case (n = 10), ascites LPA levels were higher than plasma LPA levels (not presented). Furthermore, ovarian cancer cells, but not breast cancer cells, produce LPA in response to the tumor-promoting agent PMA, suggesting that ovarian cancer cells may be the source of LPA in ascites and plasma of ovarian cancer patients (23). We thus asked whether LPA, at concentrations found in the ascites of ovarian cancer patients, could induce ovarian cancer cells to produce LPA. We incubated ovarian cancer cell lines with or without 25 µM LPA for 2 h (optimal time and concentration for LPA production as assessed in preliminary experiments) and determined the levels of LPA present in the medium. One of four ovarian cancer cell lines tested produced LPA in response to treatment with LPA (OVCA-3; see Table 1). The other three ovarian cancer cell lines

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Analysis of LPA production in unstimulated and LPA-stimulated ovarian and breast cancer cell lines*</th>
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<tr>
<td>Cell line</td>
<td>LPA produced</td>
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<tr>
<td></td>
<td>(relative PhosphorImager units)</td>
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<tr>
<td>Ovarian cancer</td>
<td></td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>199</td>
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<tr>
<td>SK-OV-3</td>
<td>7550</td>
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<tr>
<td>HEY</td>
<td>4519</td>
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<td>A2780.6.3</td>
<td>2057</td>
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<tr>
<td>Breast cancer</td>
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<tr>
<td>MCF7</td>
<td>695</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>1175</td>
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<td>MDA-MB-468</td>
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*³²P labeling and stimulation of cells, extraction of lipids, and analysis of phospholipids by two-dimensional TLC were performed as described in “Materials and Methods.” The data shown are from one of at least two or three (OVCA-3, SK-OV-3) independent experiments.
lines (SK-OV-3, HEY, and A2780.6.3) constitutively produced LPA in the absence of any exogenous stimulus, and LPA treatment did not further increase the amount of LPA produced by these cell lines (Table 1). One cell line, SK-OV-3, constitutively produced particularly high levels of LPA (Table 1 and Fig. 1A). In these cells, LPA was the predominant phospholipid released into the medium. In contrast, the breast cancer cell lines MCF7, MDA-MB-231, and MDA-MB-468 produced only low levels of LPA, and treatment with LPA did not increase LPA formation (Table 1 and Fig. 1B). Indeed, the SK-OV-3, HEY, and A2780.6.3 cell lines produced 5.6 times more LPA than the three breast cancer cell lines (4708 units versus 838 units). In contrast to the ovarian cancer cell lines tested, normal ovarian epithelial cells and immortalized ovarian epithelial cells produced very low amounts of LPA and could not be induced by LPA to produce more LPA (data not shown). The effects of LPA were specific, because lysophosphatidylcholine, EGF, and PDGF did not increase LPA production (presented herein).

Role of PLD in Constitutive LPA Formation. PLD, which converts membrane phospholipids to PA, has been implicated in LPA production by platelets. We explored the role of PLD in constitutive LPA production by SK-OV-3 cells using the ability of primary short-chain alcohols to inhibit the formation of PA by PLD (43, 44). Incubation of SK-OV-3 cells with 1-butanol at 0.5%, a concentration that completely inhibits PA formation by PLD (45), caused a consistent 50% reduction in the amount of LPA that is constitutively produced and released by SK-OV-3 cells (Fig. 2A). We conclude that there are PLD-dependent and -independent components of newly synthesized LPA release by SK-OV-3 cells. PLD-independent synthesis might involve the sequential action of PLC and diacylglycerol kinase (11).

Involvement of PLA₂ in Constitutive LPA Production. Conversion of PA to LPA by PLA₂ has been implicated in LPA synthesis by platelets. On the basis of their biological properties, PLA₂s have been classified into three subgroups: sPLA₂, cPLA₂, and iPLA₂. The relative contribution of members of each of these subgroups to LPA production is not known. We explored the role of secretory PLA₂s in the constitutive production of LPA by SK-OV-3 cells by using inhibitors of sPLA₂. SK-OV-3 cells were incubated in the presence of either mannoalide, an inhibitor of group IIA (synovial) sPLA₂ (IC₅₀, 0.02–0.2 μM; Ref. 46), or OOEPC, an inhibitor of group IB (pancreatic) sPLA₂ (IC₅₀, 6.2 μM; Ref. 47), prior to extraction and analysis of newly formed phospholipids in the medium. Mannoalide, at a concentration that inhibits group IIA sPLA₂ (48), did not alter the amount of LPA synthesized and released by SK-OV-3 cells.
which inhibits group IIA (synovial) sPLA2 (49), reduced the amount of LPA being produced and released by SK-OV-3 cells by ~80% (Fig. 2C). This result indicates that group IB sPLA2(s) play a major role in the constitutive formation of LPA by SK-OV-3 cells, whereas group IIA sPLA2 activity is not required for LPA formation in SKOV-3 cells. Both manoalide and OOEPC altered the distribution of phospholipids in cell supernatants, demonstrating efficacy of the inhibitors (not presented).

Recently, iPLA2s have been identified that display either an absolute specificity or a high selectivity for PA, therefore possibly playing a role in the induction of LPA synthesis (30). Moreover, most cell types contain cPLA2 that are specific for arachidonic acid at the sn-2 position and that potentially are also involved in LPA formation (36). The arachidonic acid analogue AACCOCF3 inhibits both cPLA2 (IC50, 50 μM; Ref. 50) and iPLA2 (IC50, 15 μM; Ref. 51) and thus can be used to explore the function of both types of cytosolic PLA2s. SK-OV-3 cells were treated with AACCOCF3 before measuring the amount of newly synthesized LPA released into the medium. AACCOCF3 at 100 μM, a concentration that completely blocks both cPLA2 and iPLA2 (52), markedly decreased the level of LPA being produced and released into the medium (90% inhibition). We conclude that cytosolic, calcium-dependent, and/or -independent PLAA2s play a critical role in the constitutive production of LPA by SK-OV-3 cells.

LPA-induced LPA Production. LPA markedly up-regulated LPA production in one of four ovarian cancer cell lines tested (OVCAR-3; see Table 1 and Fig. 3). This response was dose and time dependent. Treatment with 3 μM LPA resulted in the formation and release of a small amount of LPA. Treatment with 10 and 30 μM LPA, concentrations present in ascites of ovarian cancer patients, however, caused the production and release of substantial amounts of LPA (Fig. 4). LPA formation in response to LPA was rapid; high levels of LPA were detected in the supernatant of OVCAR-3 cells within 30 min of incubation with LPA. Very little labeled LPA could be detected after 24 h of treatment with LPA (data not shown). Ovarian cancer patients are potentially exposed to many different growth factors in ascitic fluid, among them LPA, EGF, and PDGF (12, 53). In contrast to LPA, EGF (Fig. 5), PDGF (Fig. 5), and LPC (not presented) did not increase LPA production in OVCAR-3 cells.

Role of PLD in LPA-induced LPA Formation. Pretreatment of OVCAR-3 cells with 1-butanol, which inhibits PLD-mediated PA formation, resulted in a decrease of inducible LPA production by ~60% (Fig. 6A). Because basal LPA production was also sensitive to the presence of 1-butanol (44% inhibition), PLD appears to be involved in both the induced and basal LPA production in OVCAR-3 cells. However, in each case there was also a PLD-independent component of LPA production.

Involvement of PLAA2 in LPA-induced LPA Synthesis. Both the group IIA sPLA2 inhibitor manoalide and the group IB sPLA2 inhibitor OOEPC reduced LPA-induced production of LPA by ~40% (Fig. 6B and C). This suggests that both types of secretory PLAA2 play a role in LPA-induced production. However, there remains considerable sPLA2-independent LPA-induced LPA production. We therefore assessed the participation of cPLA2 and iPLA2 in LPA-induced LPA formation by using the cPLA2 and iPLA2 inhibitor AACCOCF3. In contrast to its effect on constitutive LPA production, AACCOCF3 did not alter LPA-induced LPA production by OVCAR-3 cells (Fig. 6D). Therefore, cytosolic PLAA2s (both calcium-dependent and -independent) do not seem to participate in LPA-induced production of LPA.

LPA Receptors Implicated in LPA-induced LPA Pro-duction. We have demonstrated previously, by Northern blot analysis, that normal and immortalized ovarian epithelial cells express the LPA receptor Edg1, whereas ovarian cancer cell lines express only very low levels of Edg1 (12). mRNA levels for Edg2 markedly vary among normal and immortalized ovarian epithelial cells as well as among ovarian cancer cell lines (12, 22, 23). Edg4 mRNA is expressed in normal ovarian epithelial cells, and its mRNA levels are elevated in ovarian cancer cells (12, 22). The OVCAR-3 cell line expresses moderately increased levels of Edg4 (12, 22). Recently, a novel LPA receptor, Edg7, was identified and cloned (25). We determined its expression levels in normal and immortalized ovarian epithelial cells as well as in ovarian cancer cell lines by Northern
blot analysis. Normal and immortalized ovarian epithelial cells express barely detectable levels of Edg7 mRNA, whereas ovarian cancer cells express Edg7 at varying levels (Fig. 7). Intriguingly, the highest level of Edg7 expression is found in the OVCAR-3 cell line, which constitutively produces very low levels of LPA and which produces markedly increased levels of LPA in response to LPA. In OVCAR-3 cells, the change in Edg7 expression as compared with normal and immortalized ovarian epithelial cells is much greater than the change in Edg4 expression (Fig. 7; 19, 22). This suggests that Edg7 and potentially Edg4 may play a role in LPA-induced LPA production by OVCAR-3 cells.

DISCUSSION

LPA stimulates growth, prevents apoptosis and anoikis, decreases sensitivity to chemotherapeutic drugs, increases production of neovascularization mediators, and increases invasiveness of ovarian cancer cells (12). LPA levels are elevated in ascites and plasma from ovarian cancer patients, implicating it in ovarian tumorigenesis (2–6). Because LPA levels are higher in ascitic fluid than in plasma, it has been hypothesized that LPA is produced in the peritoneal cavity and then migrates to the peripheral circulation. Ovarian cancer cells have been implicated to be the source of LPA production in ascites, because they have been shown to synthesize LPA in response to the tumor-promoting agent PMA (28). However, whether PMA mimics a physiological process is not known. Ovarian cancer cells in the patient might produce LPA either constitutively or after activation by the cellular milieu. Knowledge of regulation of LPA production by ovarian cancer cells and the enzymes involved in LPA synthesis could lead to the development of therapeutic measures that would interfere with LPA synthesis and its deleterious effects on ovarian cancer cells. Here, we report that three of four ovarian cancer cell lines tested constitutively produce LPA at much higher levels than breast cancer cells or normal ovarian epithelial cells, and that one ovarian cancer cell line can be induced by LPA to produce LPA. Strikingly, the one ovarian cancer cell line induced to release LPA by LPA constitutively produced the lowest level of LPA, raising the possibility that constitutive LPA production by the other ovarian cancer cell lines played a role in amplifying LPA production. Normal ovarian epithelial cells, which constitutively produces LPA, has been implicated as a possible source of LPA in ascites. However, whether this constitutive production is a physiological process is not known. Knowledge of regulation of LPA production by ovarian cancer cells and the enzymes involved in LPA synthesis could lead to the development of therapeutic measures that would interfere with LPA synthesis and its deleterious effects on ovarian cancer cells.
produce low levels of LPA, did not produce LPA in response to exogenous LPA. Normal ovarian epithelial cells express low levels of mRNA for the Edg4 and 7 LPA receptors (12, 22). mRNA levels for Edg4 and particularly Edg7 are markedly elevated in ovarian cancer cells (12, 22), suggesting that the novel expression of these receptors may mediate LPA-induced LPA production by ovarian cancer cells. Levels of Edg1 mRNA, a putative LPA receptor, are high in normal ovarian epithelial cells but low in most ovarian cancer cells (12, 22), suggesting that this receptor is not relevant to LPA-induced LPA production. Edg2 levels are not consistently altered between normal ovarian epithelial cells and cancer cells (12, 22, 23), and furthermore, Edg2 appears to function as a negative receptor for LPA in ovarian cancer cells (23).

The two main types of enzymes involved in LPA synthesis are PLD, which contains at least two isoforms, and PLA2, which contains at least 10 different isoforms. PLD is involved in the formation of the LPA precursor PA, and as shown herein, PLD indeed plays a role in the production of LPA in ovarian cancer cells. Little is known about the role that the various PLA2 enzymes play in LPA formation. It has been shown that sPLA2 is inactive on intact membrane bilayers but requires membrane rearrangement and subsequent loss of membrane asymmetry to mediate LPA production (35, 54–56). Such loss of membrane asymmetry occurs during apoptosis or malignant transformation (57). We have shown that LPA production by ovarian cancer cells requires group IB (pancreatic) sPLA2 activity, whereas group IIA (synovial) sPLA2 does not seem to play a role in constitutive LPA production by ovarian cancer cells and seems to play only a minor role in the induction of LPA by LPA. There seems to be a differential requirement for cPLA2 and/or iPLA2 phospholipase A2 by cells that constitutively produce LPA versus cells that are induced by LPA to produce LPA.
seems to be a mutual interdependency between sPLA2 and cPLA2, because group IIA sPLA2 also increases the expression of cPLA2 (60). Recently, it was reported that sPLA2 may indirectly regulate cPLA2 by activating p38MAPK (61), which in turn phosphorylates cPLA2, contributing to its activation (42, 62). Taken together, these findings point to a complex interplay between sPLA2 and cPLA2, possibly also iPLA2. Specific PLA2 isofrom inhibitors should allow further elucidation of the effects that the various PLA2 enzymes display on each other and on specific functions, such as LPA synthesis. LPA has been previously demonstrated to activate PLD (39 – 41). The mechanism(s) by which LPA regulates PLAs have not, however, been explored. We have demonstrated previously that LPA induces rapid increases in cytosolic free calcium and activates MAPK in ovarian cancer cells (13). It was thus somewhat surprising that cPLA2, which is activated by increases in cytosolic calcium (42), does not seem to be involved in LPA-induced LPA production. We have shown recently that LPA activates p38MAPK in OVCAR-3 cells. This might be a mechanism by which constitutively produced LPA in ovarian cancer cells contributes to cPLA2 activation.

PLA1, which cleaves at the sn-1 position of a glycerophospholipid, may be involved in the production of one particular species of LPA found in the ascites of ovarian cancer patients. LPA found in ascites consists of a mixture of sn-1 and sn-2 species, with the sn-2 species exhibiting greater bioactivity than the sn-1 counterpart (4). PA, the precursor of LPA, is the preferred substrate of both a membrane-bound (63) and a cytosolic PLA1 (64), thus further implicating PLA1 in LPA synthesis. It will be interesting to explore the contribution of PLA1 to LPA production by ovarian cancer cells once inhibitors of PLA1 become available. Interestingly, a recently described isoform of sPLA2, sPLA2-β, prefers sn-1 cleavage to sn-2 cleavage (65), whereas another isoform, sPLA2-γ, efficiently cleaves at both positions (66). These cPLA2 enzymes could thus also contribute to sn-2 LPA formation in the ascites of ovarian cancer patients.

LPA levels in cell membranes are low (11), reflecting rapid conversion or degradation of LPA. Reduced rates of conversion and/or degradation might contribute to the elevated levels of newly synthesized LPA in the supernatant of ovarian cancer cells as compared with breast cancer cells. This may also contribute to LPA-induced increases in LPA levels. LPA is converted back to PA by LPA acyltransferase, whereas PA phosphohydrolases and lysophospholipases rapidly degrade LPA (11). Decreased expression or activity of these enzymes may contribute to the increased LPA levels in ovarian cancer patients.

In summary, we have shown that ovarian cancer cells, but not breast cancer cells or normal ovarian epithelial cells, release high levels of LPA into the extracellular medium. We have further shown that PLD plays a role in LPA synthesis by ovarian cancer cells, and that different PLAs isoforms are required for constitutive and LPA-induced LPA production. These findings are clinically relevant because ascites and plasma of ovarian cancer patients, but not of patients with nongynecological tumors, contain elevated levels of LPA. LPA in ovarian cancer patients might be used as a marker for early diagnosis and as a molecular target for therapeutic intervention.
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