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Analysis of Phosphatidylcholines (PCs) and Lysophosphatidylcholines (LysoPCs) in Metastasis of Breast Cancer Cells*

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Abstract The important role of phospholipids in many processes including cell cycle regulation, inflammation and tumorigenesis is increasingly becoming the focus of research. Previously we found that cytosolic phospholipase A2 (cPLA2) is over-expressed in breast cancer cells. Here, we assessed the profiles of phosphatidylcholines (PCs) and lysophosphatidylcholines (LysoPCs) that were mediated by cPLA2 in breast cancer cells. MCF-7 and LM-MCF-7 (with high metastatic potential) cell lines were used as models and the profiles of phospholipids were analyzed by HPLC/ESI/MS^n. Our results showed that the levels of 10 species of LysoPCs were higher in LM-MCF-7 cells than that in MCF-7 cells. Meanwhile, six species of PCs that may produce arachidonic acid (AA) and associated LysoPCs were lower in LM-MCF-7 cells than that in MCF-7 cells. Furthermore, we identified that 4 out of 6 species of PCs were responsible for the generation of AA mediated by cPLA2 in LM-MCF-7 cells. The increased ratio of LysoPCs to PCs corresponds to the activation level of cPLA2. Thus, we conclude that 4 identified species of PCs and 4 corresponding species of LysoPCs mediated by cPLA2, as well as the up-regulation and activation of cPLA2, may involve in the metastasis of breast cancer cells.

Key words phosphatidylcholine (PC), lysophosphatidylcholine (LysoPC), cytosolic phospholipase A2 (cPLA2), breast cancer
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Recent studies have demonstrated that arachidonic acid (AA) metabolism is associated with cancer development[1–3]. AA is an important constituent of membrane phospholipids that can be liberated by cytosolic phospholipase A2 (cPLA2) activation. The important role of phospholipids in proliferation and migration of cancer cells is increasingly becoming the focus of research[4–7]. Phosphatidylcholines (PCs) are the major phospholipids found in the membranes of mammalian cells. The endogenous PCs are deacylated by cPLA2 to generate AA and lysophosphatidylcholines (LysoPCs). LysoPCs have inflammatory activities and activates signaling molecules including tyrosine kinases[8–10]. The binding of LysoPCs to their receptors may regulate signaling pathways including inflammation and cell migration[8, 11–12]. Analysis of profile alterations of the highly heterogeneous

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mixtures of lipids provides a good way for understanding the complex functions of lipids.

Previously, our group established a high metastatic subclone from breast cancer MCF-7 cell lines, designated LM-MCF-7, which had high malignant phenotype in cell proliferation and migration [13-18]. Using these two cell lines we demonstrated that AA metabolites were involved in proliferation and migration of breast cancer cells, in which the enzymes associated with AA metabolism including cPLA2, cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX) and 12-LOX were over-expressed [19]. However, the species of PCs hydrolyzed into AA and corresponding LysoPCs by cPLA2 in breast cancer progression remain poorly understood.

Although high performance liquid chromatography (HPLC) allows the separation and quantification of many classes of phospholipids, the information of the molecular species of each class still requires more elaborate techniques. HPLC with ultraviolet (UV) is not useful for the detection of some important classes of lipids such as lysolipids because of low UV absorbance. Tandem electrospray ionization mass spectrometry (ESI/MS) possesses high sensitivity and affords highly reproducible results[20-22]. Although it is known that the composition of phospholipids affects cancer metastasis[7, 23-25], details regarding the component and molecular species of phospholipids that affect tumor metastasis remain unclear due to the limitations of experimental approach.

In the present study, we focus on the investigation of metabolites of phospholipids involving metastasis of breast cancer cells by HPLC/ESI/MS^n. Our data showed that 4 identified species of PCs and 4 corresponding species of LysoPCs mediated by cPLA2 were altered in cPLA2-overexpressed LM-MCF-7 cells. This finding provides new insight into the function of PCs and LysoPCs in the metastasis of breast cancer cells.

1 Materials and methods

1.1 Cell culture

MCF-7 and LM-MCF-7 cells were maintained in RPMI 1640 medium (Gibco, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, CA, USA), 100 U/ml penicillin, 100 mg/L streptomycin and 1% glutamine. The cells were incubated at 37℃ in a humidified atmosphere with 5% CO_2.

1.2 Treatment of tumour cells

MCF-7 and LM-MCF-7 cells were cultured in 150 mm culture plates at a density of 2×10^7 cells/plate. LM-MCF-7 cells were re-cultured in serum-free medium for 12 h, followed by treatment with 20 μmol/L nordihydroguaiaretic acid (NDGA, Sigma, St. Louis, MO, USA, an inhibitor of 5-LOX), 20 μmo/L indomethacin (Indo, Sigma, St. Louis, MO, USA, an inhibitor of COX-2) or both for 24 h. MCF-7, LM-MCF-7 cells and treated LM-MCF-7 cells were used for Western blot analysis and HPLC/ESI/MS^n analysis.

1.3 Western blot analysis

Western blot analysis was performed as described previously [19]. Primary antibodies used were anti-β-actin (Sigma, St. Louis, MO, USA) and anti-phospho-cPLA2 (Cell Signaling Technology, Beverly, MA, USA). Horseradish peroxidase-linked anti-rabbit or anti-mouse antibody was used as the secondary antibody. The membrane was developed with SuperSignal Ultra (Pierce, Rockford, IL, USA).

1.4 Lipid extraction and phospholipid analysis by HPLC/ESI/MS^n

MCF-7, LM-MCF-7 and LM-MCF-7 cells treated with both NDGA and Indo were collected and then the lipids were extracted by the modified Bligh and Dyer method [26]. A combined qualitative and quantitative analysis of phospholipids was carried out on a Finnigan LC/ESI/MS^n system consisting of a Surveyor HPLC with quaternary gradient pumps and an auto-sampler coupled with Finnigan LCQ Advantage ion-trap mass spectrometer (Thermo Electron, San Jose, CA, USA), or on a LC/ESI/MS system consisting of a Waters 600E HPLC with an auto-sampler coupled with a Micromass Quattro micro API triple-quadruple mass spectrometer (Micromass, Manchester, UK), as previously described by Yang *et al* [27], with minor changes.

1.5 Statistical analysis

Statistical analysis was performed using Sigma Plot 2001 (Systat Software Inc., Richmond, CA, http://www.systat.com). Each experiment was repeated at least three times. Statistical significance was assessed by comparing mean values ($\bar{x} \pm s$) using a Student's t test. A P-value of less than 0.05 was regarded as statistically significant.

2 Results

2.1 Major LysoPCs in MCF-7 and LM-MCF-7 cells

Phospholipids are associated with many processes including tumorigenesis[7, 28]. Here, we are interested in alteration of phosphatidylcholines (PCs) and their metabolites lysophosphatidylcholines (LysoPCs) in metastasis of breast cancer cells. Using negative-ion HPLC/ESI/MS analysis, the base peak chromatogram of LysoPCs from MCF-7 and LM-MCF-7 cells showed that the level of LysoPCs (1.43×10^4) of LM-MCF-7 cells was higher than that of MCF-7 cells (9.89×10^3) (Figure 1a, b). We next examined which specific molecular species of LysoPCs were increased. The LysoPCs, having a choline group at the polar head, were detected as chloride adducts $[M+Cl]^-$. The MS^1 spectrum showed molecular species of LysoPCs by HPLC/ESI/MS^n analysis in LM-MCF-7 cells, in which each m/z presented one or more species (Figure 1c). To further identify these molecular species of LysoPCs in detail, we directly infused the samples into the source and detected those using class-specific MS^n (MS^2 or MS^3) scan modes. We obtained the composition and structural information of 12 LysoPCs from MCF-7 and LM-MCF-7 cells (Table 1). Noticeably, our data revealed that the levels of 10 out of 12 LysoPCs were significantly higher in LM-MCF-7 cells relative to that of MCF-7 cells (Table 1, Figure 1d). Thus, our data suggest that the increase of the 10 LysoPCs is involved in the metastasis of breast cancer cells.

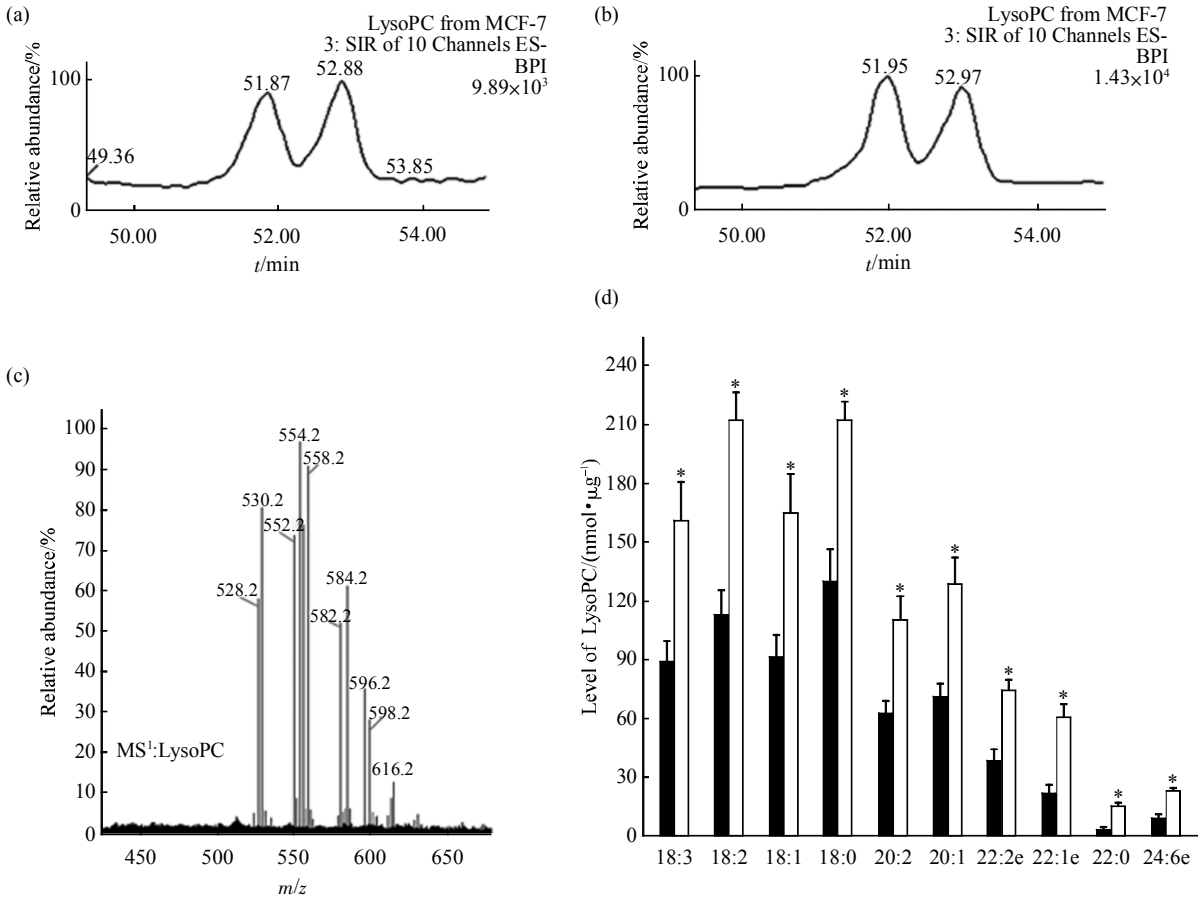

Fig. 1 Major LysoPCs in MCF-7 and LM-MCF-7 cells

Negative-ion HPLC/ESI/MS base peak chromatogram of LysoPCs from (a) MCF-7 cells and (b) LM-MCF-7 cells. (c) The MS^1 spectrum of LysoPCs molecular species from LM-MCF-7 cells by HPLC/ESI/MS^n analysis. (d) Histogram shows profiles of LysoPCs in MCF-7 and LM-MCF-7 cells. ■: MCF-7; □: LM-MCF-7. Data are represented as the mean±SD of four different observations and compared to MCF-7 cells using Student's t-test. *P < 0.01 *vs* MCF-7 cells was considered significant.

Table 1 Molecular species and quantification of LysoPCs in MCF-7 and LM-MCF-7 cells

$[M-X]^-$ (m/z)	Molecular species	Amount/(nmol•μg^{-1})	
		MCF-7	LM-MCF-7
528.2	16:1	134.60±11.25	125.46±14.12
530.2	16:0	171.70±18.58	170.55±19.09
552.2	18:3	89.73±10.12	161.34±19.78*
554.2	18:2	113.24±18.23	212.78±21.89*
556.2	18:1	92.29±19.11	165.02±20.54*
558.2	18:0	130.40±16.52	202.43±10.16*
582.2	20:2	63.12±6.16	110.66±15.07*
584.2	20:1	71.54±6.58	128.78±17.89*
596.2	22:2e	38.96±5.95	74.50±5.57*
598.2	22:1e	22.43±4.12	60.76±7.08*
614.2	22:0	4.18±0.76	15.76±1.21*
616.2	24:6e	9.35±1.24	23.49±1.53*

*P < 0.01 *vs* MCF-7 cells, Student's t test.

2.2 Identification of PCs involving release of arachidonic acid and LysoPCs

We have reported that arachidonic acid (AA) metabolites were involved in proliferation and migration of breast cancer cells, in which the enzymes associated with AA metabolism including cPLA2, cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX) and 12-LOX were over-expressed [19]. To identify PCs that may be responsible for the generation of AA and LysoPCs in breast cancer cells, we examined the levels and structures of 10 PCs (named 838.5, 840.5, 842.5, 844.5, 868.5, 870.5, 882.5, 884.5, 900.5 and 902.5) that may produce AA and 10 corresponding LysoPCs. Using negative-ion HPLC/ESI/MS analysis, the base peak chromatogram of PCs from MCF-7 and LM-MCF-7 cells showed that levels of PCs were lower in LM-MCF-7 cells (PC level: 1.33×10^5) relative to MCF-7 cells (PC level: 1.74×10^5) (Figure 2a, b). We next analyzed specific molecular species of PCs in the metastatic LM-MCF-7 cells. The averaged mass spectra interpretation was shown by negative fragment ions (Figure 2c). To characterize the molecular species of these PCs in detail, the samples were directly infused into the source and detected by class-specific MS^n (MS^2 or MS^3) scan modes. We identified the structures and compositions of 10 PCs from MCF-7 and LM-MCF-7 cells (Table 2). Moreover, our data revealed that the levels of 6 out of 10 molecular

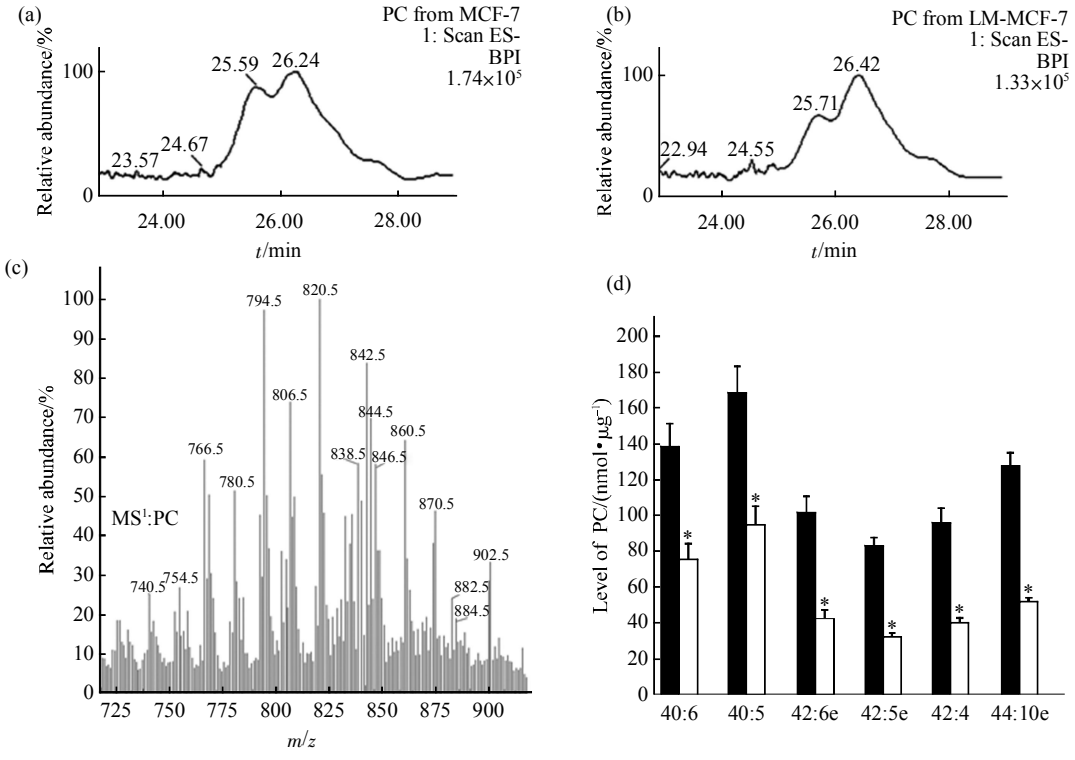

Fig. 2 Identification of PCs involving release of arachidonic acid and LysoPCs

Negative-ion HPLC/ESI/MS base peak chromatogram of PCs from (a) MCF-7 cells and (b) LM-MCF-7 cells. (c) MS^1 spectrum of PCs molecular species from LM-MCF-7 cells by HPLC/ESI/MS^n analysis. (d) Histogram shows the 7 molecular species of PCs in MCF-7 and LM-MCF-7 cells. ■: MCF-7; □: LM-MCF-7. Data are represented as the mean±SD of four different observations and compared to MCF-7 cells using Student's t-test. *P < 0.01 *vs* MCF-7 cells was considered significant.

species of PCs were lower in LM-MCF-7 cells relative to MCF-7 cells (Table 2, Figure 2d). Structure analysis by MS^2 and MS^3 further demonstrated that the Sn-2 position of these 6 molecular species of PCs was 20:4 (arachidonic acid, 20:4), suggesting that these PCs(40:6, 40:5, 42:6e, 42:5e, 42:4, 44:10e) might be responsible for the generation of arachidonic acid.

Table 2 Molecular species and quantification of PCs in MCF-7 and LM-MCF-7 cells

$[M-X]^-$ (m/z)	Molecular species Sn-1/Sn-2	Amount/(nmol•μg^{-1})	
		MCF-7	LM-MCF-7
838.5	18:0e/20:0	213.78±13.38	237.06±20.85
840.5	18:3/20:3	176.38±19.88	180.25±20.87
842.5	18:2/20:3	310.54±20.65	330.08±31.57
844.5	18:1/20:3	265.87±27.59	281.25±25.62
868.5	20:2/20:4	138.89±12.89	75.21±9.26*
870.5	20:1/20:4	168.48±14.85	95.40±10.77*
882.5	22:2e/20:4	102.13±9.26	42.50±5.12*
884.5	22:1e/20:4	83.29±4.16	32.39±2.32*
900.5	22:0/20:4	96.11±9.31	40.26±2.61*
902.5	24:6e/20:4	128.14±7.67	51.82±2.99*

*$P < 0.01$ *vs* MCF-7 cells, Student's *t* test.

2.3 Identification of PCs involved in the release of arachidonic acid mediated by cPLA2

COX-2 and 5-LOX are involved in the AA metabolism. We previously reported that the inhibition of COX-2 and 5-LOX mediated by Indo and NDGA was able to result in down-regulation of cPLA2 through reducing the level of p-ERK1/2[19]. Accordingly, to clarify the corresponding species of PCs that can be hydrolyzed by cPLA2 to generate AA and LysoPC in LM-MCF-7 cells, we examined the structure and composition of PCs and LysoPCs using HPLC/ESI/MS^n when the levels of p-cPLA2 were decreased in LM-MCF-7 cells by treatment with Indo and NDGA. Western blot analysis showed that the levels of p-cPLA2 were decreased in LM-MCF-7 cells treated with both Indo and NDGA (Figure 3a, b). Using negative-ion HPLC/ESI/MS analysis, the base peak chromatograms from LM-MCF-7 and LM-MCF-7 cells treated with both Indo and NDGA showed that the levesl of LysoPCs were decreased from 1.43×10^4 to 1.18×10^4 and the levels of PCs were increased from 1.33×10^5 to 1.69×10^5 when the level of p-cPLA2 was reduced (Figure 3c～f).

To further identify the species of PCs that were hydrolyzed by cPLA2 into AA and LysoPC, we examined the structure and composition of above identified 6 PCs and 6 LysoPCs in LM-MCF-7 cells and LM-MCF-7 cells treated with both Indo and NDGA by HPLC/ESI/MS^n analysis. The results showed that the levels of 4 PCs (42:6e, 42:5e, 42:4, 44:10e) were increased and corresponding 4 LysoPCs (22:2e, 22:1e, 22:0, 24:6e) were decreased in LM-MCF-7 cells when the level of p-cPLA2 was decreased (Table 3, Figure 3g, h), suggesting that cPLA2 might hydrolyze these 4 PCs into AA and their corresponding LysoPCs.

2.4 Increased ratio of LysoPC to PC represents the activation level of cPLA2, which may be involved in the metastasis of breast cancer cells

Next, we analyzed the ratio of LysoPC to PC (LysoPC22:2e/PC42:6e, LysoPC22:1e/PC42:5e, LysoPC22:0/PC42:4 and LysoPC24:6e/PC44:10e in MCF-7, LM-MCF-7 and LM-MCF-7 cells treated with Indo and NDGA (Table 4). We observed that the ratio of LysoPC to PC of LM-MCF-7 cells was higher than that of MCF-7 cells. Noticeably, this ratio was reduced in LM-MCF-7 cells after the levels of p-cPLA2 were decreased by treatment with Indo and NDGA (Table 4, Figure 4), suggesting that the ratio of LysoPC to PC represents the enzymatic activity of cPLA2 [29–31]. The molecular species of PC884, identified by their negative fragment ions, MS^2 spectrum of m/z 884.50, and MS^3 spectrum of m/z 834.36 were shown in Figure 5. For the chloride adduct of PCs (m/z 884.5), the single major MS^2 fragment ion was observed from the loss of a methyl group and chloride. MS^3 of m/z 834.36 showed the 548.12, 530.17 and 302.98 values corresponded to the $[M-15-C_{18}H_{30}CHCO]^-$, $[M-15-C_{19}H_{31}COOH]^-$ and $[C_{19}H_{31}COO]^-$ fragments, respectively. Thus, the PC m/z 884 is PC 22:1e/20:4. The alkyl fragment in sn-1 position could not be seen in MS^2 and was not charged, and perhaps not even formed. Thus, we conclude that the increased ratio of LysoPC to PC mediated by the activation of cPLA2 may be involved in the metastasis of breast cancer cells.

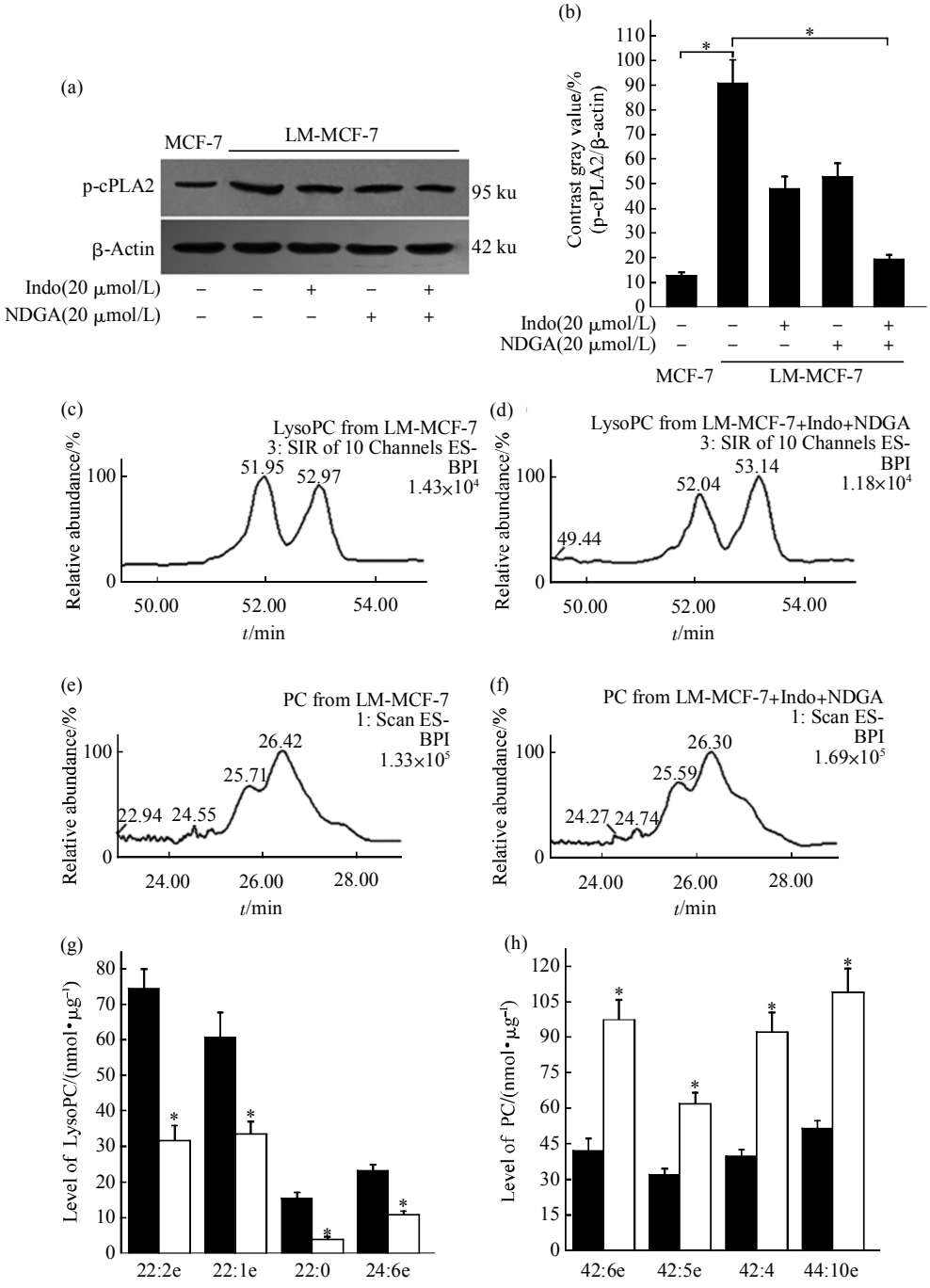

Fig. 3　Identification of PCs involved in the release of arachidonic acid mediated by cPLA2

(a, b) The levels of p-cPLA2 were tested by Western blot analysis in MCF-7 and LM-MCF-7 cells treated with Indo, NDGA, or both. (c, d) Negative-ion HPLC/ESI/MS base peak chromatogram of LysoPCs of LM-MCF-7 cells and LM-MCF-7 cells treated with both Indo and NDGA were shown. (e, f) Negative-ion HPLC/ESI/MS base peak chromatogram of PCs of LM-MCF-7 cells and LM-MCF-7 cells treated with both Indo and NDGA were shown. (g, h) Histogram shows the 4 molecular species of LysoPCs (c, d) and PCs (e, f) in LM-MCF-7 cells and LM-MCF-7 cells treated with Indo and NDGA. Data are represented as the mean±SD of four different observations and compared to LM-MCF-7 cells using Student's t-test. *$P < 0.01$ vs LM-MCF-7 cells was considered significant. ■: LM-MCF-7; □ : LM-MCF-7+Indo+NDGA.

Table 3 Molecular species and quantification of LysoPCs and PCs in LM-MCF-7 cells and LM-MCF-7 cells treated with both Indo and NDGA

PL	$[M-X]^-$ (m/z)	Molecular species Sn-1/Sn-2	Amount/(nmol•μg^{-1})	
			LM-MCF-7	LM-MCF-7+Indo+NDGA
LysoPC	582.2	20:2	110.66±15.07	103.36±16.59
	584.2	20:1	128.78±17.89	120.36±18.04
	596.2	22:2e	74.50±5.57	31.67±4.27*
	598.2	22:1e	60.76±7.08	33.64±3.86*
	614.2	22:0	15.76±1.21	3.89±0.72*
	616.2	24:6e	23.49±1.53	10.90±1.25*
PC	868.5	20:2/20:4	75.21±9.26	81.48±12.57
	870.5	20:1/20:4	95.42±10.77	109.14±14.87
	882.5	22:2e/20:4	42.53±5.12	97.66±8.77*
	884.5	22:1e/20:4	32.39±2.32	61.72±5.17*
	900.5	22:0/20:4	40.26±2.61	92.66±8.27*
	902.5	24:6e/20:4	51.82±2.99	109.48±9.95*

*P < 0.01 vs LM-MCF-7, Student's t test.

Table 4 The ratio of LysoPC to PC in breast cancer cells

Name	Ratio (LysoPC/PC)		
	MCF-7	LM-MCF-7	LM-MCF-7+Indo+NDGA
LysoPC22:2e/PC42:6e	0.38±0.05	1.75±0.15*	0.32±0.05§
LysoPC22:1e/PC42:5e	0.26±0.04	1.87±0.13*	0.54±0.06§
LysoPC22:0/PC42:4	0.04±0.007	0.39±0.02*	0.04±0.009§
LysoPC24:6e/PC44:10e	0.07±0.02	0.45±0.03*	0.09±0.03§

*P < 0.01 vs MCF-7 cells, Student's t test. §P < 0.01 vs LM-MCF-7 cells, Student's t test.

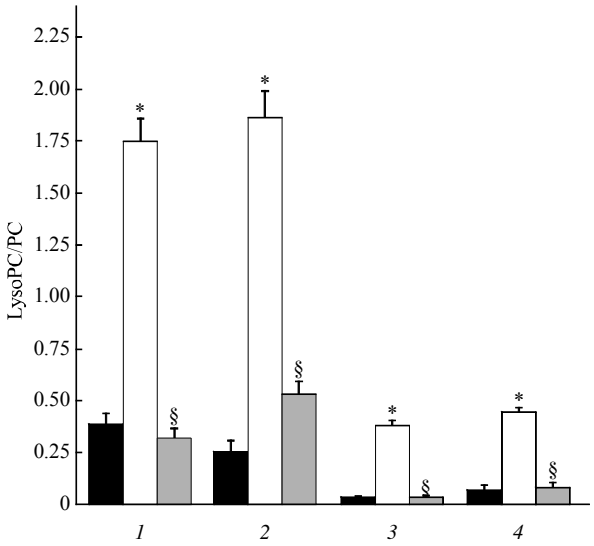

Fig. 4 Increased ratio of LysoPC to PC represents the activation level of cPLA2, which may be involved in the metastasis of breast cancer cells

Histogram shows the ratio of LysoPC to PC in MCF-7, LM-MCF-7 cells and LM-MCF-7 cells treated with both of Indo and NDGA. Data are represented as the mean ±SD of four different observations using Student's t-test. *P < 0.01 vs MCF-7 cells, §P < 0.01 vs LM-MCF-7 cells were considered significant. ■: MCF-7 ; □: LM-MCF-7; ▨: LM-MCF-7 +Indo +NDGA. *1*: LysoPC22:2e/PC42:6e; *2*: LysoPC22:1e/PC42:5e; *3*: LysoPC22:0/PC42:4; *4*: LysoPC22:6e/PC44:10e.

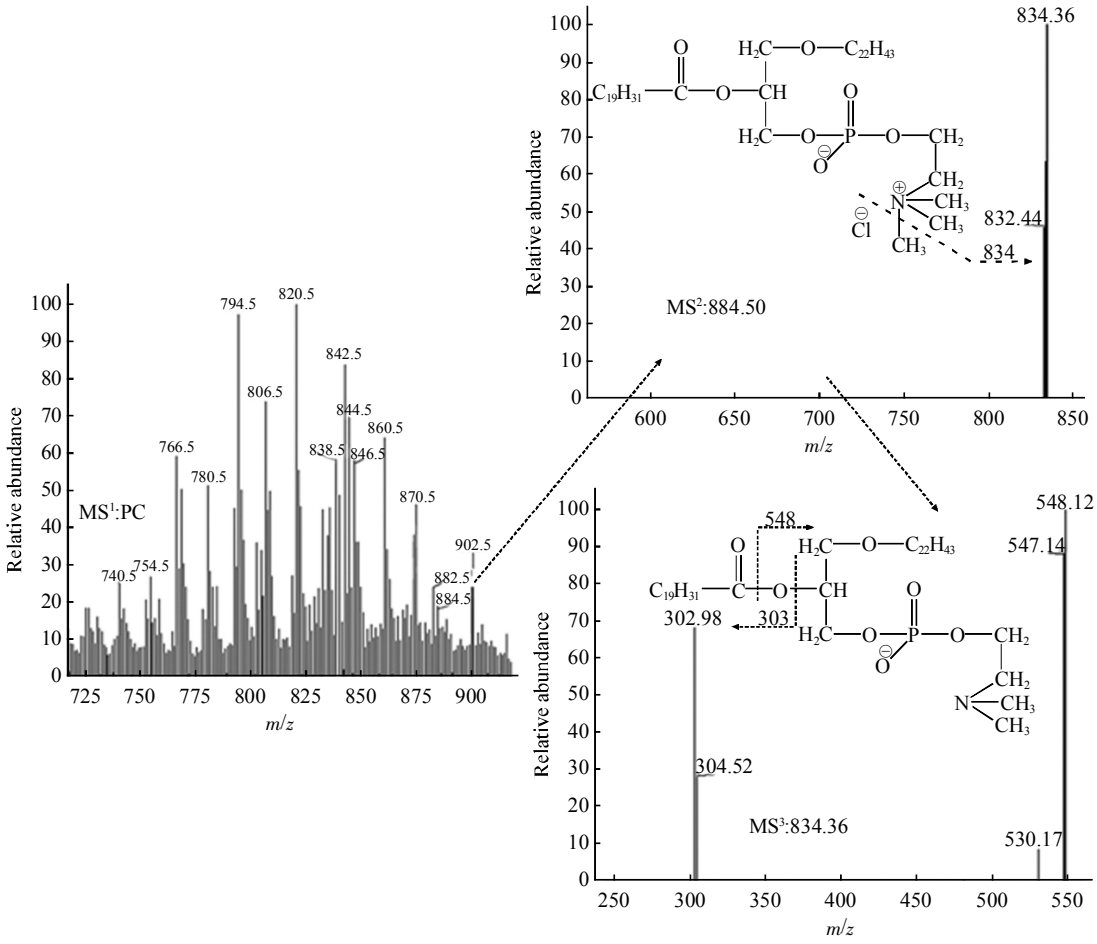

Fig. 5 Analysis of structure of PC884 by Negative-ion LC/ESI/MS^n

Averaged mass spectrum of PCs was shown in LM-MCF-7 cells. MS^2 spectrum of *m/z* 884.50 and MS^3 spectrum of *m/z* 834.36 were shown. PC *m/z* 884 is PC 22:1e/20:4.

3 Discussion

Functions of lipids are critical in malignant tumors as they are necessary not only for providing the membrane constituents of proliferating cells but also for signalling pathways that drive tumorigenesis [28, 32]. However, the details in which the specific components and molecular species of phospholipids involved in the development of breast cancer remain unclear.

In the present study, we are interested in analysis of phospholipids profiles involved in the metastasis of breast cancer cells. We focused on the identification of the metastasis-associated PCs and LysoPCs that were mediated by cPLA2 in MCF-7 and LM-MCF-7 cells by HPLC/ESI/MS^n analysis. We first examined the major LysoPCs in MCF-7 and LM-MCF-7 cells. The levels of 10 identified LysoPCs were altered in the metastatic LM-MCF-7 cells (Figure 1, Table 1). It has been reported that AA metabolism are correlated with cancer development [1-2]. Our group has demonstrated that AA metabolites were involved in proliferation and migration of breast cancer cells [19]. Accordingly, to identify the PCs that are responsible for the generation of AA and LysoPCs in breast cancer cells, we examined the compositions and structures of the 10 corresponding PCs in MCF-7 and LM-MCF-7 cells by HPLC/ESI/MS^n analysis. We found that only 6 out of 10 PCs were lower in LM-MCF-7 cells relative to MCF-7 cells (Figure 2, Table 2). Moreover, MS^2 and MS^3 analysis showed that the Sn-2 of the 6 PCs was 20:4, which corresponds to AA (20:4) (Table 2). It suggests that the 6 PCs could be hydrolyzed into AA

and corresponding LysoPCs in MCF-7 and LM-MCF-7 cells. However, the Sn-2 of the remaining 4 PCs with no difference between MCF-7 and LM-MCF-7 cell lines was 20:0 or 20:3 which is not the value of AA. It suggests that the hydrolysis of these 4 PCs does not directly generate AA in MCF-7 and LM-MCF-7 cells.

We have reported that LM-MCF-7 cells with high metastatic potential exhibited high levels of the enzymes of AA metabolism including cPLA2. To further determine the species of PCs that can be hydrolyzed by cPLA2, to generate AA and LysoPCs in LM-MCF-7 cells, we analyzed the species of 6 identified PCs and LysoPCs by HPLC/ESI/MS^n analysis when p-cPLA2 was decreased by treatment with both Indo and NDGA. We found that the levels of 4 out of 6 PCs (42:6e, 42:5e, 42:4 and 44:10e) were increased in LM-MCF-7 cells treated with Indo and NDGA. The corresponding LysoPCs from these 4 PCs (22:2e, 22:1e, 22:0 and 24:6e) were decreased in LM-MCF-7 cells when the level of p-cPLA2 was decreased by treatment with Indo and NDGA(Figure 3, Table 3). Thus, we conclude that cPLA2 hydrolyzes these 4 PCs (22:2e/20:4, 22:1e/20:4, 22:0/20:4 and 24:6e/20:4) into AA and their corresponding LysoPCs in LM-MCF-7 cells.

We further examined the enzymatic activity of cPLA2 by determining the ratio of LysoPC to PC in breast cancer cells (Figure 4, Table 4). The results showed that the ratio of LysoPC to PC in LM-MCF-7 cells was higher relative to MCF-7 cells. It suggests that the enzymatic activity of cPLA2 is higher in LM-MCF-7 than that in MCF-7 cells. The decrease of levels of p-cPLA2 mediated by Indo and NDGA was able to reduce the ratio of LysoPC to PC in LM-MCF-7 cells, which is consistent with the Western blot analysis (Figure 3a). Thus, we conclude that the enzymatic activity of cPLA2 is higher in breast cancer cells with high metastasis potential, suggesting that cPLA2 is closely involved in tumor metastasis. It has been reported that phospholipids profiles can be used for potential differential diagnosis of human cerebral cancer, lung cancer, prostate cancer and esophageal squamous cell carcinoma [33-36]. However, the reports only identified the alterations of total phospholipids. Up to date, the identified molecular species of PCs and LysoPCs in the metastasis of breast cancer cells remain largely unexplored.

In summary, we assessed the corresponding metabolites of phospholipids mediated by cPLA2 in MCF-7/LM-MCF-7 breast cancer cells using HPLC/ESI/MS^n. Our results showed that the levels of 10 species of LysoPCs were higher in LM-MCF-7 cells than that in MCF-7 cells. Six species of PCs were responsible for the generation of AA, of which 4 were responsible for the generation of AA mediated by cPLA2 in LM-MCF-7 cells. The increased ratio of LysoPCs to PCs corresponds to the activation of cPLA2. Together, cPLA2 associated LysoPCs and PCs may be involved in the metastasis of breast cancer cells and potentially serves as prognostic biomarkers in cancers. Our finding provides new insights into the role of aberrant lipid metabolism in the metastasis of breast cancer.

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乳腺癌转移中的磷脂酰胆碱和溶血磷脂酰胆碱分析 *

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摘要 我们以前曾报道花生四烯酸(arachidonic acid，AA)代谢产物可以促进乳腺癌细胞增殖和迁移．为了进一步寻找维持高转移乳腺癌细胞中 AA 高水平代谢的内源机制，深入探求 AA 代谢促进乳腺癌细胞转移的分子机理，我们应用 HPLC/ESI/MS^n 技术检测和分析了乳腺癌 MCF-7 和高转移乳腺癌 LM-MCF-7 细胞中溶血磷脂酰胆碱(lysophosphatidylcholines，LysoPCs)和磷脂酰胆碱(phosphatidylcholines，PCs)的成分和含量．发现了 10 种 LysoPC 的含量在 LM-MCF-7 细胞中显著高于 MCF-7 细胞，有 6 种 PC 可水解产生 AA，它们在 LM-MCF-7 细胞中的含量显著低于 MCF-7 细胞，提示这些溶血磷脂含量的升高和磷脂含量的降低可能与乳腺癌转移相关．在 LM-MCF-7 细胞中，COX-2 抑制剂吲哚美辛(indomethacin，Indo)和 LOX 抑制剂(nordihydroguaiaretic acid，NDGA)共同作用可明显下调 cPLA2 的活性，应用 HPLC-ESI-MS^n 技术比较 cPLA2 活性下调前后 LM-MCF-7 细胞中 LysoPC 和 PC 含量的变化，发现其中 4 种 PC 可被 cPLA2 水解产生 AA．还发现，细胞内 LysoPC 与 PC 的比值可以反映 cPLA2 的活性．通过以上研究进一步证实了由 cPLA2 活性调节的 AA 释放及代谢对乳腺癌转移具有重要作用．

关键词 磷脂酰胆碱，溶血磷脂酰胆碱，cPLA2，乳腺癌
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