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Fatty Acid Compositions of Phosphatidylserine, Phosphatidylinositol and Cardiolipin in Human Breast Cancer and Reference Tissues

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Abbreviations

PC,phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PS, phosphatidylserine; PI, phosphatidylinositol; CL, cardiolipin; 18:0-20:4, 1-stearoyl-2-arachidonoyl; TLC, thin layer chromatography; GLC, gas liquid chromatography

ABSTRACT

There are hardly any reports regarding the fatty acid compositions of phosphatidylserine (PS), phosphatidylinositol (PI), and cardiolipin (CL) in human breast cancer. Here, we compared the fatty acid compositions of PS, PI and CL in breast cancer tissues with those in reference tissues.

It was found that 18:0 and 18:1 characteristically occupied over 80% of the fatty acid composition of breast tissue PS. In breast cancer tissues relative to the reference tissues, saturated fatty acid was increased from 57.7% to 66.3% while 20:4 was decreased from 30.2% to 18.6% in PI, 18:0 was increased from 35.5% to 43.2% while 18:1 was decreased from 43.7% to 37.2% in PS, and 18:1 was increased from 9.7% to 25.5% while 18:2 was decreased from 60.7% to 50.1% in CL.

Key words; human, breast, cancer, fatty acid, phosphatidylserine, phosphatidylinositol, cardiolipin, stearic acid, oleic acid

Membrane phospholipids are necessary for maintenance of membrane structure and functions [1-3]. Prostaglandin E2 derived from arachidonic acid of membrane phospholipids suppresses the immune system and stimulates the growth of epithelial cells [4-6]. Chajés *et al.* suggested that tumor-membrane fatty acids may influence several steps of carcinogenesis, and it therefore appears important to determine which factors may influence membrane fatty acids [7].

On the other hand, although the roles of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in breast cancer have been studied [8-10] and there are many reports

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regarding the fatty acid compositions of PC, PE and phosphatidylinositol (PI) in human breast cancer [6,7,11-13], fatty acid analyses of minor phospholipids, such as phosphatidylserine (PS) and cardiolipin (CL), have hardly been reported to date. In the present study, we analyzed the fatty acid compositions of these phospholipids for the first time, and found characteristic changes in the fatty acid compositions of these minor phospholipids in breast cancer.

EXPERIMENTAL PROCEDURES

Patients and tissues

Breast tissue samples were obtained from 10 females patients with breast cancer undergoing surgery at the Kurashiki Medical Center. Part of each tumor sample was used for histology and the remainder was used for the present study. All 10 patients (mean age, 52.3 ± 17.6 years; age range, 29~87 years) gave informed consent for the study according to the requirements of the hospital committees. The clinical stages of the patients were stage I in 1 patient and stage II in 9 patients.

A non-cancerous glandular tissue (reference sample) was dissected from normal tissue of the same mammary gland for each patient. The cancer and reference tissues were freed from adipose tissue and immediately frozen in liquid nitrogen.

Lipid Analysis

The tissues (0.4 ~ 1.6g of cancer and 0.4~ 3.1g of reference tissues) were homogenized in 10 - 15mL of methano-chloroform (2 : 1, v/v) containing 0.01% butylated hydroxytoluene, and then crude lipids were extracted by the method of Bligh and Dyer [14], in which water was replaced with 2% NaCl. As described previously [15], the phospholipids were fractionated from crude lipids by passage through a silica Sep Pak cartridge. Analysis the phospholipid compositions by TLC and the fatty acid compositions of these phospholipids by GLC were described in the previous papers [16,17]

All results were expressed as means ± SD. Student's t-test for equal variance and the Aspin-Welch t-test for unequal variance were used for comparison of cancer and reference tissues. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The total phosholipid-phosphorus (PL-P) contents were 368.6 ± 88.6 and 125.2 ± 77.3 μg/g of tissue for the cancer and reference tissues, respectively (Table 1). In other words, the mean PL-P content was 3-fold higher in the cancer tissues than in the reference tissues, consistent with previous reports [11,12].

We detected seven phospholipid species in our TLC analysis. The proportion of PE was significantly higher in the cancer tissues than in reference tissues ($P<0.05$), while that of sphingomyelin (SM) was significantly lower in the cancer tissues than in reference tissues ($P<0.05$).

The proportions of n-6 polyunsaturated fatty acids in cancer tissue PC and PE were

decreased by about 8% compared with those in the reference tissues, and in particular, the linoleic acid (18:2) of PC was decreased by about 50% (Table 2). In contrast, the proportions of palimitic (16:0) and oleic (18:1) acids in PC, and of stearic acid (18:0) and 18:1 in PE in cancer tissues were all increased. On the other hand, aldehyde 16:0 and aldehyde 18:0 occupied about 20% of the

Table 1. Phospholipid Compositions of Cancer and Reference Tissues[a].

	Phospholipid-phosphorus	Phospholipid Composition (wt%)						
	μg/tissue	lyso PC	SM	PC	PI	PS	PE	CL
Cancer	368.6±88.6*	0.7±0.2	11.1±1.8*	50.8±3.0	5.8±0.9	4.5±1.7	26.0±2.3*	1.4±0.5
Reference	125.2±77.3	0.8±0.5	15.8±2.1	50.0±2.5	6.1±1.4	3.4±1.3	22.7±3.2	1.3±1.2

[a]Data are presented as means ± SD for ten samples.
*Significantly different from reference, P<0.05
Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PS, phosphatidylserine; PI, phosphatidylinositol; CL, cardiolipin.

Table 2. Fatty acid compositions of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and cardiolipin (CL) in breast cancer and reference tissues[a] (%).

Fatty Acids	PC		PE		PS		PI		CL	
	Cancer	Reference	Cancer	Reference	Cancer	Reference	Cancer	Reference	Cancer	Reference
Saturated										
16:0ad[b)]	1.0±0.3*[c)]	1.8±0.7	13.6±3.0	15.1±3.3	0.1±0.1	0.3±0.3	nd[d)]	nd	0.5±0.3	1.5±1.3
16:0	39.4±9.6	35.0±2.7	6.5±2.7	4.5±1.2	7.8±3.7	5.6±1.7	9.2±3.7	5.0±0.9	7.3±4.2	6.8±2.7
18:0ad	nd	nd	5.5±2.3	7.1±5.6	nd	nd	nd	nd	0.2±0.2	0.2±0.3
18:0	9.2±1.5	9.5±1.7	18.9±2.2*	12.4±3.6	43.2±3.2*	35.5±2.5	56.5±4.2	51.4±3.6	2.1±2.7	3.4±3.8
Total[e)]	50.8	46.9	44.7	39.3	52.1	41.8	66.3	57.7	12.4	13.9
Monounsaturated										
16:1	0.4±1.2	nd	nd	0.9±0.6	0.8±0.9	1.8±0.9	0.5±0.6	1.0±1.2	5.1±2.0	5.4±2.0
18:1	31.0±6.2	27.1±2.7	24.4±4.4*	15.8±1.8	37.2±3.4*	43.7±4.4	4.2±4.4	3.1±4.5	25.5±4.7*	9.7±5.7
Total[f)]	32.1	27.9	26.5	18.5	38.8	46.5	5.3	4.4	30.9	15.7
n-6Polyunsaturated										
18:2	9.0±4.3*	16.3±1.6	5.8±3.0	8.6±3.6	4.6±1.1	7.3±2.3	2.4±1.3	2.3±1.5	50.1±9.0*	60.7±9.2
20:4	5.4±1.9	6.0±1.3	14.7±4.0*	24.4±1.7	0.9±1.1	1.4±1.2	18.6±8.9*	30.2±9.9	0.3±0.3	1.4±0.7
Total[g)]	15.6	23	21.6	33.9	7.3	9.8	25.6	35.4	53.4	63.8
n-3Polyunsaturated										
22:6	1.1±0.3	1.5±0.8	5.3±1.6	5.6±1.1	1.5±1.7	1.5±0.7	2.1±1.7	2.0±1.2	3.1±1.1	5.4±2.0
Total[h)]	1.5	2.2	7.2	8.3	1.8	1.9	2.8	2.5	3.3	6.6

a) Data are presented as means ± SD for ten samples.
b) Dimethylacetal derivatives of aldehydes.
c) *Significantly different from reference tissues, P<0.05.
d) nd : not detected
e) Including 12:0, 14:0, 20:0. f) Including 17:1, 20:1.
g) Including 20:2, 20:3. h) Including 18:3, 20:5, 22:5.

plasmalogen PE.

The major fatty acids in PI were 18:0 and arachidonic acid (20:4). The proportion of 20:4 in cancer tissue PI was remarkably decreased compared with that in the reference tissues, whereas the saturated fatty acid content was increased.

The major fatty acids in PS were 18:0 and 18:1, which occupied 80% of the fatty acids. 18:0 was increased in the cancer tissues compared with the reference tissues, while 18:1 was decreased. This phenomenon differed from the results for PC, PE, PI and CL.

The major fatty acid in CL was 18:2. The proportion of 18:2 in the cancer tissue CL was remarkably decreased compared with that in reference tissues, whereas 18:1 was increased.

As mentioned in other reports [7,11,13], the proportions of n-6 polyunsaturated fatty acids in the cancer tissue PC and PE were decreased compared with those in reference tissues. Such changes in the fatty acid compositions of PC and PE, which occupy 70%~80% of the total phospholipid, may influence the membrane fluidity and the activities of membrane enzyme [1].

PI is an important functional phospholipid that takes part in signal transduction [2,18]. It was estimated that 18:0-20:4 PI occupied 60% of the PI, since 20:4 occupied 30% of the PI in the reference tissues. However, it was estimated that 18:0-20:4 PI decreased to 37% in the cancer tissues, which would create a problem for signal transduction in cancer cells.

Breast PS composed of 18:0 and 18:1 may have an important physiological function. For example, PS is essential for protein kinase C activity in cancer cells [19], and externalization of the inner membrane PS is important for apotosis [3,20]. 18:1 only decreased in PS in the cancer tissues, and was rather increased in the other phospholipids. The changes in the 18:0 and 18:1 proportions in PS may be related to the physiological functions, as mentioned above.

CL containing 60% 18:2 is an essential phospholipid for the activity of cytochrome oxidase, which is associated with the energy production system of the mitochondra [21]. Therefore, the decrease in 18:2 and increase in 18:1 observed in breast cancer tissues will influence the energy production system.

Taken together, the characteristic changes in the fatty acid compositions of PS, PI, and CL in human breast cancer may be of physiological significance.

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ヒト乳ガンのホスファチジルセリン、ホスファチジルイノシトールおよびカルジオリピンの脂肪酸組成

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要約

ヒト乳ガンのリン脂質であるホスファチジルセリン（ＰＳ）、ホスファチジルイノシトール（ＰＩ）およびカルジオリピン（ＣＬ）の脂肪酸組成についてはほとんど報告がない。我々は、これらのリン脂質の脂肪酸組成を対照組織のそれらと比較した。

その結果、乳組織のホスファチジルセリンの脂肪酸は、ステアリン酸（18：０）とオレイン酸（18：１）が全体の80%をも占めていた。

乳癌組織と対照（非乳癌組織）を比べると、ＰＩでは飽和脂肪酸酸が57.7から66.3%に癌組織では増加し、逆にアラキドン酸は30.2から18.6%に減少した。PSでは、18：０が35.5から43.2%に癌組織で増加し、逆に18：１は43.7から37.2%に減少した。一方、ＣＬでは、18：１は9.7から25.5%に癌組織で増加し、逆にリノール酸は60.7から50.1%に減少した。

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