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Acyl Group Composition of Membrane Phospholipids in Mammary Tissues and Carcinoma Induced by Dimethylbenz(a)anthracene[1] (39465)

BENJAMIN S. LEUNG AND GRACE Y. SUN

Department of Surgery and Clinical Research Center, University of Oregon Medical School, Portland, Oregon 97201 and Sinclair Comparative Medicine Research Farm, University of Missouri, Columbia, Missouri 65201

In recent years, considerable effort has been directed toward understanding the role of fatty acids in membrane functions (1). Membrane-bound hormone receptors, for example, the prolactin receptor molecule, have been reported to contain a phospholipid fraction (2). Changes in fatty acid composition may alter the structural integrity of membrane, and possibly also affect the binding activity of membrane-bound receptor sites of hormones. These changes may also modify the transport of critical nutrients inside the cell, thereby promoting the manifestation of a neoplastic state (3). Results from our laboratory (4) and from White (5) showed that acyl group composition of individual phospholipids in subcellular fractions of human brain tumors exhibited a pattern different from the corresponding fractions of normal brain tissues. By comparing hepatomas with host liver tissues, other investigators (6, 7), also demonstrated changes in the acyl group patterns of tumors. The present investigation was undertaken to see whether similar changes may occur in mammary tumors of rats induced by DMBA as compared to normal breast tissues. Since DMBA-tumors are estrogen-prolactin dependent (8), it may be possible to establish a relationship between the hormonal effect on tumor growth and changes in composition of phospholipids in these tumors.

Materials and Methods. Induction of tumor. Tumors were induced in 50-day-old Sprague-Dawley rats (Holtzman Co., Madison, Wis.) by a single intragastric feeding of 16 mg of DMBA (Sigma Chemical Co., St Louis, Mo.), suspended in sesame oil. In about 6 weeks, tumors began to develop. The presence of estrogen receptors, which is a characteristic of estrogen-dependent tissues (9, 10), was detected in all the tumors in this study. These tumors were also identified histologically as adenocarcinoma. Portions of the tumors were frozen for lipid analysis. Breast tissues were isolated from tumor-bearing rats either contralaterally to the tumors or, if ipsilaterally, at a remote distance from the tumors.

After the connective tissues, skin or visible necrotic areas were removed, and the tumors or normal breast tissues were weighed and minced with scissors. A 4-vol to tumor-weight of Tris-EDTA buffer (10 m*M* Tris and 1.5 m*M* EDTA, pH 7.5) was added. The suspension was homogenized with a polytron PT-10 tissue disintegrator (Brinkmann Instrument, Westbury, N.Y.) for two 15-sec periods with a 30-sec cooling interval. The resulting homogenate was centrifuged for 15 min at 3500*g* to remove cellular debris and red blood cells. The supernatant solution was used for the extraction of lipids.

Analysis of membrane lipids. Tissue lipids were extracted with chloroform-methanol, 2:1 (v/v) (11). Neutral glycerides were separated from phosphoglycerides by Unisil column elution (12). Neutral glycerides and other nonpolar lipid materials were eluted by passing 50 ml of chloroform through a column packed with 2 g of Unisil. Phospholipids and other polar lipids were eluted with 100 ml of methanol. Individual neutral glycerides and phosphoglycerides were further separated by tlc. The thin-layer plates were prepared with Silica gel G suspended in 0.01 *M* Na_2CO_3 (Brinkmann Instruments, Westbury, N.Y.). Triglycerides were separated by one-dimensional tlc, using hex-

[1] Abbreviations used: Diacyl-GPC, diacyl-glycerophosphorylcholine; diacyl-GPE, diacyl-glycerophosphorylethanolamine; diacyl-GPI, diacyl-glycerophosphorylinositol; diacyl-GPS, diacyl-glycerophosphorylserine; tlc, thin-layer chromatography; glc, gas-liquid chromatography; DMBA, 7,12-dimethylbenz(a)anthracene.

ane:diethyl ether:15 *N* ammonium hydroxide 70:30:0.1 (v/v) as solvent for development. Phospholipids were subjected to separation-reaction-separation by tlc as described by Horrocks and Sun (11), except that the solvent system for the second dimension was modified (13). After solvent development, lipid spots were visualized by exposing the thin-layer plates to iodine vapor, as in the case to be used for phosphorus assay. When the lipid spots were required for fatty acid analysis, the thin-layer plates were sprayed with ethanolic 2′,7′-dichlorofluorescein. Individual lipid spots were scraped into test tubes and the amount of lipid-phosphorus was determined according to Gottfried (14). For the analysis of acyl group composition of the phospholipids and triglycerides, lipids were subjected to alkaline methanolysis (15). Methyl esters of fatty acid were analyzed by a gas chromatograph fitted with a digital integrator (Hewlett Packard). Repeated analyses of the same sample showed good reproducibility to within 5% variation of the peak area.

Results. Compositions of phospholipids in breast tissues and tumors from DMBA-treated animals are shown in Fig. 1. Approximately 40% of the phospholipids of the control breast tissues were diacyl-GPC. Sphingomyelin, alkenylacyl-GPE and diacyl-GPE each constituting about 10 to 20%. Cardiolipins were present in breast tissue and tumors in small amounts (2-5%) but were not analyzed because the lipid spot was not completely separated from the free fatty acid spot in the thin-layer system. In the DMBA-tumors there was an increase in the proportion of alkenylacyl-GPE, diacyl-GPE, and diacyl-GPI and a decrease in the proportion of diacyl-GPC as compared to controls. A small proportion of monoacyl-GPC was also present in some breast tissues and tumors, but there was no apparent alteration in the proportion of this phosphoglyceride in the tumor tissue. The value of the ratio diacyl-GPE/diacyl-GPC of tumors was about two times higher than that of the normal breast tissues.

Phosphoglycerides of breast tissues and tumors were characterized by their distinct patterns of fatty acids (Fig. 2). Acyl groups of diacyl-GPC were high in 16:1 and 18:1; diacyl-GPE in 18:0, 18:1, and 20:4; diacyl-

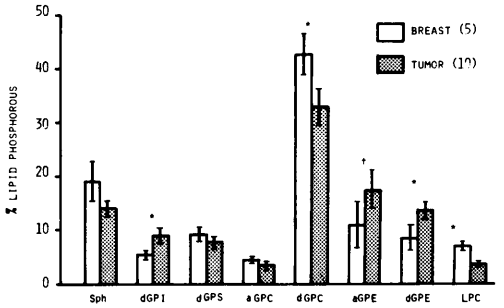

FIG. 1. Membrane phospholipid composition of DMBA-tumor and mammary tissues. Methods of membrane preparation and quantitation of phospholipids were described in the text. Values (mean ± SD) are expressed as percentages of total lipid phosphorus content. Ten tumor samples and five mammary glands of the same tumor-bearing animals were analyzed, respectively. Only four tumors have measurable LPC fractions. Abbreviations: Sph, sphingomyelin; dGPI, diacyl-GPI, dGPS, diacyl-GPS; aGPC, alkenylacyl-GPC; dGPC, diacyl-GPC; aGPE, alkenylacyl-GPE; dGPE, diacyl-GPE, LPC, monoacyl-GPC; * and † show statistical significance of $p < 0.001$ and 0.005, respectively, by the student's *t* test (tumor vs control).

GPI in 18:0 and 20:4; and diacyl-GPS in 18:0 and 18:1. In contrast, acyl groups of alkenylacyl-GPE contained mainly 20:4. Acyl group composition of phosphoglycerides in adenocarcinoma had a higher proportion of 18:1, and a lower proportion of 18:2 than breast tissues. Moreover, an increase in the proportion of 16:0 was also observed in some phosphoglycerides of tumors. There was no apparent change in the proportion of 20:4 (*n*-6) among other phosphoglycerides (Fig. 2A-C) except for a slight decrease of 20:4 (*n*-6) in diacyl-GPI in tumors (Fig. 2D). Although quantitative changes in fatty acids were demonstrated, each individual phospholipid in tumor tissue appeared to maintain the same distinct acyl group profile as in normal tissue.

About 60% of the fatty acids of alkenylacyl-GPE was 20:4 (*n*-6), but the proportion of this fatty acid was not altered in the neoplastic tissue (Fig. 3A). Consequently, acyl group composition of alkenylacyl-GPE in tumors remained essentially the same as in breast tissues. Triglycerides of breast tissues and tumors were high in 16:0, 18:1, and 18:2 fatty acids (Fig. 3B). In contrast to phosphoglycerides, there was no remarkable variation in acyl group pattern of triglyc-

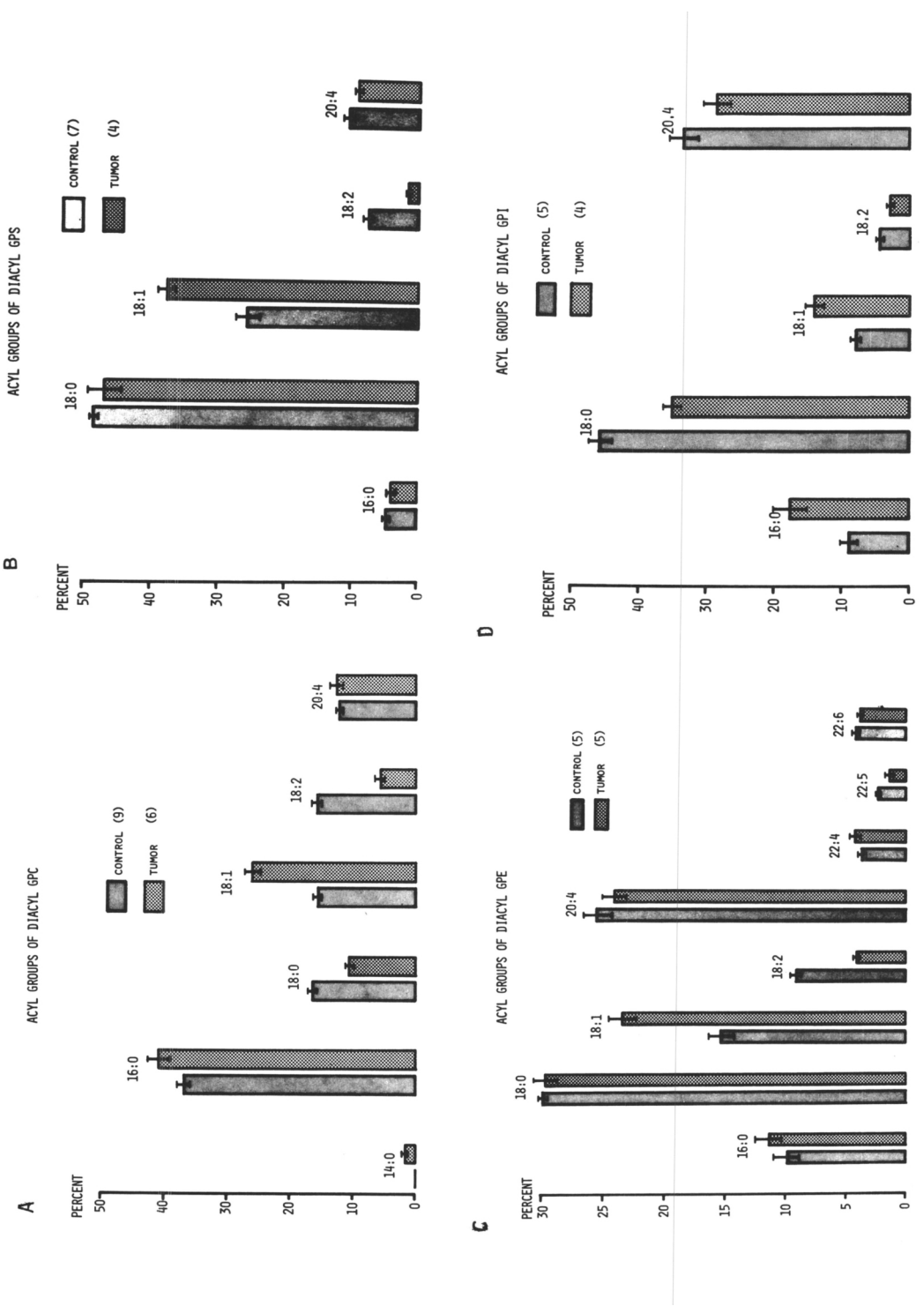

FIG. 2. The acyl group compositions of individual phosphoglycerides in DMBA-tumor and breast tissues. Isolation of membrane phospholipids and determination of fatty acid by glc are as described in materials and methods. Values (mean ± SEM) are expressed as weight percents according to peak areas of glc. The number of determinations are as indicated in parentheses of the chart.

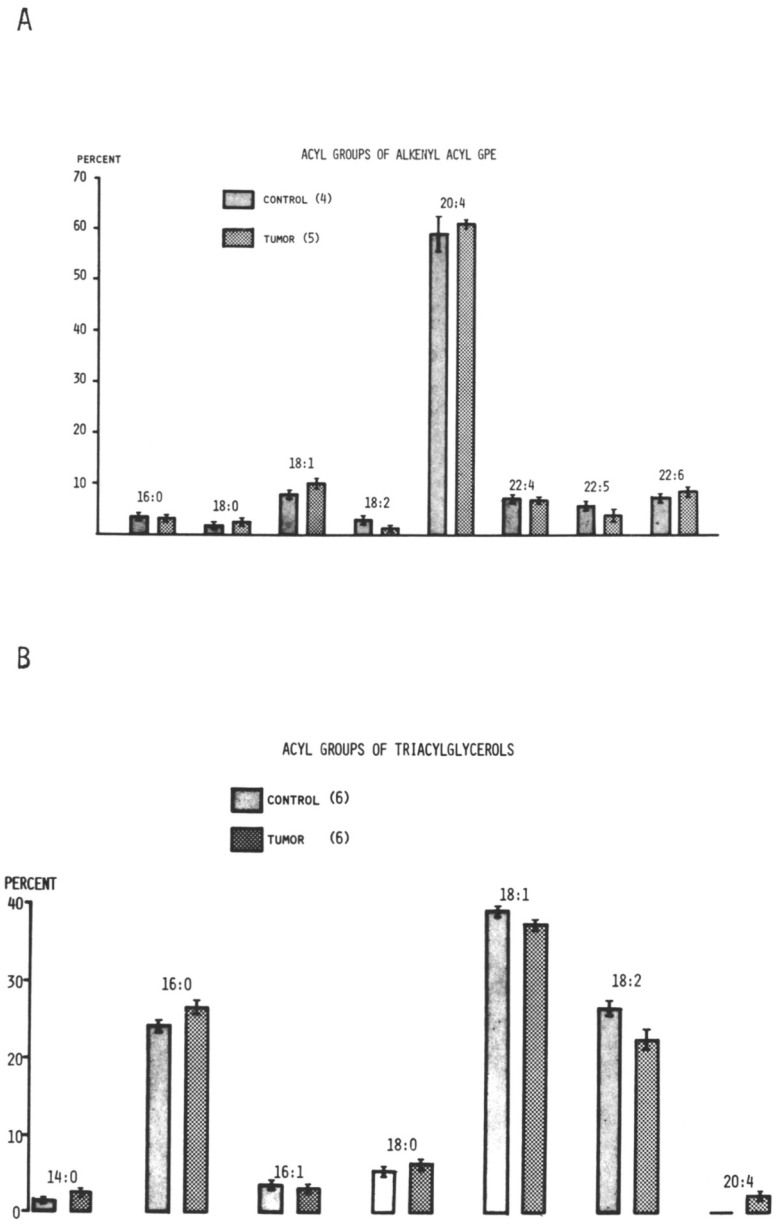

FIG. 3. The acyl group compositions of alkenylacyl-GPE (A) and of triglycerides (B) in tumors and breast tissues. See Fig. 2 for details.

erides between breast and tumor tissues.

When the relative amounts of 20:4 to 18:2 of the various phosphoglycerides for breast and tumor tissues were evaluated (Table I), an elevation of the ratio of phosphoglycerides for tumors was observed. The increase in ratio was accounted for largely by the decrease in the proportion of 18:2 in tumors.

Analyses of phospholipids and fatty acids were also performed with normal breast tissues obtained from rats without DMBA treatment and at various periods during pregnancy and lactation (16). These studies showed changes in phospholipid and acyl group compositions in breast tissue during different physiological states. However, the lipid composition between breast tissue

TABLE I. RATIO OF 20:4/18:2 IN BREAST TISSUES AND TUMORS OF DMBA TREATED RATS.[a]

	dGPI	aGPE	dGPC	dGPE	dGPS
Breast tissue	7.6	19.9	0.8	2.8	1.3
DMBA-tumor	9.8	47.8	2.2	5.8	5.1

[a] Ratio of 20:4/18:2 was calculated from the fatty acid content of each individual phospholipids as shown in Figs. 2 and 3.

from normal rats and from DMBA-treated rats was similar. Hence, breast tissue of DMBA-treated animals was selected as control tissue for comparison.

Discussion. Changes in phospholipids may be a common abnormality of cellular membranes in transformed cells (17). Differences in composition of phospholipids between DMBA-tumors and control breast tissues were demonstrated in the present study. Tumor tissue had a decrease in diacyl-GPC and an increase in diacyl-GPE, alkenylacyl-GPE and, diacyl-GPI. Since comparisons were made on pieces of normal and tumor tissues and not on specific cell types, it is possible that the normal and neoplastic tissue samples contained different proportions of blood and connective tissue. Therefore, some of the observed changes could reflect tissue compositional differences rather than those due to mammary tumors, *per se*. However, histological examinations have revealed that the tumor specimens were composed primarily of adenocarcinoma cells, suggesting phospholipid and fatty acid changes were due to the cancer cells.

Differences in acyl group profiles of individual phospholipids between DMBA-tumors and breast tissues were demonstrated in this study. Although both tissue and tumors contained a high proportion of triglycerides, fatty acid changes in tumors were more specific for phospholipids than for triglycerides. The most marked change was the decrease in the proportion of 18:2 in the phosphoglycerides of tumor. Since 18:2 is an essential fatty acid which is supplied exogenously to the cells rather than synthesized by the cells, reduction of 18:2 in phospholipids may reflect a decrease in the utilization of this fatty acid as a structural component for cellular membrane. In the present experiment, the decrease in the proportion of 18:2 was compensated largely by the increase of 18:1. This condition was similar to that observed during chronic deficiency of essential fatty acid (18), except that the characteristic increase of 20:3 (*n*-9) in tissues was not noted in breast tumors. Possibly there was an inadequate supply of the nutrient fatty acids in tumor cells, resulting in changes similar to that of a localized deficiency of essential fatty acid.

Changes in acyl group composition of phosphoglycerides have been reported for a number of hepatomas in comparison to fatty acid composition of the respective normal tissues (6, 7). In most tumors examined, there was an increase in the proportion of 18:1 in the major phosphoglycerides but a decrease of 18:2 was reported only in Morris Hepatoma (5123C) (7). In general, a decrease in the proportion of 20:4 (*n*-6) was also noted in these tumors. Brain tumors have been reported to exhibit an increase in 20:4 (*n*-6) and a decrease in 22:6 (*n*-3) when compared to normal brain tissues (4). The cause for acyl group changes among different tumors is not known. However, these changes may be related to many cellular abnormalities since the acyl groups of phosphoglycerides are not only structural ingredients of membranes but are also important in regulating different cellular functions.

The turnover of 18:2 with respect to its conversion to 20:4 may have been altered during neoplastic transformation. Since 20:4 (*n*-6) is a precursor for the biosynthesis of prostaglandins F_2 and E_2 (19), the decrease of 18:2 and 20:4 in diacyl-GPI of tumor might reflect a more rapid biosynthesis of prostaglandin. Indeed, Tan *et al.* (20) have recently demonstrated a higher concentration of prostaglandin E_2 and a relatively more rapid conversion of labeled arachidonic acid to prostaglandin in DMBA-tumors than in normal breast tissue.

In conclusion, characteristic phospholipid profiles and their side chain fatty acids in breast tissues were altered in neoplasm. Changes in fatty acid composition were similar among phospholipids. There was no apparent change in fatty acids in triglycerides. It is suggested that an altered rate of metabolism of the essential fatty acid, 18:2, may have occurred in tumors. Modifications of

phospholipids in the membrane could, in turn, contribute to abnormal functions or differentiation of cells.

Summary. Phospholipids and their acyl group composition in mammary adenocarcinomas and mammary tissue of the same tumor-bearing animals were investigated. Breast adenocarcinoma induced by dimethylbenz(*a*)anthracene exhibited a phospholipid pattern which was different from that of the mammary tissue. Tumor phospholipids had higher proportions of diacyl-GPI, diacyl-GPE, and alkenylacyl-GPE and a lower proportion of diacyl-GPC than the controls. The acyl groups of most phospholipids in tumors showed a marked increase in the proportion of 18:1 and a decrease in the proportion of 18:2. The fatty acid composition of plasmalogen and triglyceride, however, remained unchanged. In spite of the decrease in the proportion of 18:2, there was no apparent difference in the proportion of 20:4 in most of the phosphoglycerides; however, a significant decrease in this fatty acid was noted in diacyl-GPI. Results of this study demonstrated that the membrane phospholipids of mammary adenocarcinoma were altered in respect to acyl group composition. Changes in physical properties of the cell membrane, in turn, could lead to abnormal manifestation of membrane regulated events in tumor cells.

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