CANCER RESEARCH

Cell Membrane Fatty Acid Composition Differs Between Normal and Malignant Cell Lines

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Twenty-eight fatty acids (C8:0 to C24:l n-9) were measured by gas chromatography in four normal cell lines (C3H / 10T1 / 2, CCD-18Co, CCD-25SK and CCD-37Lu) and seven cancer cell lines (C-41, Caov-3, LS-180, PC-3, SK-MEL-28, SK-MES-1 and U-87 MG). Results show differences in the content and proportions of fatty acids when comparing cancer cell lines with their normal counterparts. Cancer cell lines showed lower C20: 4 n-6, C24:1 n-9, polyunsaturated fatty acids (PUFA's) and ratios of C20:4 n-6 to C20:5 n-3 and C16:0 to C18:1 n-9 and stearic to oleic (SA/OA) than their normal counterparts. All cancer cell lines had SA / OA ratios lower than 7.0 while normal cell lines had ratios greater than 0.7 ($p<0.05$). In addition, the ratios of total saturated fatty acids (SFA) to PUFA'S and the concentration of C18:1 n-9, C18:2 n-6, C20:5 n-3 were higher in cancer cell lines as compared to normal cell lines. A positive correlation was detected between C16:0 and longer SFA'S ($r=+0.511$, $p<0.05$) in normal cell lines whereas a negative correlation ($r=0.608$, $p<0.05$) was obtained for malignant cell lines. Moreover, cancerous cell lines exhibited a particular desaturation defect and an abnormal incorporation of C18:2 n-6 and C20-4 n-6 fatty acids.

Key words: Fatty acid composition, Cell membrane and cancer

It is well recognized that exogenous fatty acids may be toxic to cultured cells at high concentrations[1]. In blood, the highest solubility of free fatty acids is ~ 1μm above this concentration free fatty acids may act as detergents and disrupt protein and membrane architecture. Saturated fatty acids can be synthesized from acetyl-CoA and malonyl-CoA up to palmitic acid, then elongated and desaturated to longer chain fatty acids. Although mammalian systems possess four desaturases ("9, "6, "5 and " 4); they are unable to insert double bonds into positions beyond " 9.

Previous in vitro studies have demonstrated that the capacity of various polyunsaturated fatty acids (PUFA'S) of killing cancer cells was associated to their ability to generate free radicals that stimulate the production of secondary products of lipid peroxidation (2,3). These studies have been further validated in an in vivo system utilizing human mammary carcinoma cell lines (MDA-MB 231 and MCF-7) transplanted to nude mice (4, 5). This growth inhibitory PUFA effect can be blocked by the addition of antioxidants such as vitamin E (5). However, one study showed that C20:5 n-3 had 60% cell growth inhibition of human lung, breast and prostate carcinoma cells, while C22:6 n-3 had only 30% (6). In another study C12:0 and C16:0 inhibited the growth of colon cancer cells (HT-29), in more significant manner than 18:2 n-6 and this inhibitory action was not blocked by vitamin E (7). These studies suggest yet another mechanism in addition to lipid peroxidation that may be operating in the fatty acid effect upon cell proliferation.

Differences in prostaglandin metabolism have been suggested (8). Nevertheless it seems paradoxical that the same fatty acid could be either a promoter of tumorigenesis and/or an antitumor agent. It has been reported that linoleic acid (LA), an essential fatty acid, can be an effective promoter of mammary tumorigenesis (9). In contrast, it has also been reported that LA suppressed malignant cell line proliferation in culture (10). While we have tackled this issue previously (11), we speculate that fatty acids influence upon cell metabolism can be different in different types of cell. We also believe that by balancing fatty

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acids (Omega 3 and 6) in the cell, we may positively influence membrane transport, energy and prostaglandin metabolism. Therefore by modifying cell membrane fatty acid composition we may be capable of modulating many aspects of cell metabolism including cell proliferation. In this way it becomes relevant to know the fatty acid composition of these normal and malignant cell lines frequently used in our laboratories. In this study four normal and seven malignant cell lines were analyzed for fatty acid composition.

Materials and Methods

Cell lines and culture. C3H / 10T½ (mouse embryo fibroblast), CCD-18Co (human colon fibroblast), CCD-25SK, CCD-37Lu (human normal lung fibroblast), C-41 (human cervical carcinoma), Caov-3 (human ovarian adenocarcinoma), LS-180 (human colon adenocarcinoma), PC-3 (human prostate adenocarcinoma), SK-MEL-28 (human malignant melanoma), SK-MES-I (human lung squamous carcinoma) and U-87 MG (human glioblastoma-astrocytoma) were obtained from American Type Culture Collection (Rockville, MD). Stock cells were grown in 75 cm^2 and 150 cm^2 poly-styrene monolayer tissue culture flasks (corning) containing bicarbonate-buffered Dulbecco's" modified Eagle media supplemented with 10% (V/V) heat inactivated (56°C for 30 min) Fetal Calf Serum (FCS, Hy Clone Laboratories, Logan, UT), 2mM glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin, 110 mg/ml sodium pyruvate, and 2.5 μg/ml. Fungi zone (experimental growth medium, EGM). Cells were incubated at 37°C in a humidified, 5% oxygen and 95% CO_2 atmosphere. Cell lines were routinely tested for mycoplasma contamination using Mycotrim-TC (Irvine Scientific, Santa Ana, CA). Cell counting was performed with a model ZM coulter counter (Coulter Electronics, Hialeah, FL) or by a Neubauer hemocytometer. The gain and threshold settings on the Coulter counter were selected for each line by matching the results to those of the hemocytometer.

Fatty acid analysis. The method for lipid extraction was done according to Folch (12) (RBC-3, 13) with slight modifications. Briefly, the cells were harvested at about 8 million or more and washed twice with 0.9% saline (NS). The cell pellets were stored at –70°C until use. To the cell pellets, 0.5ml of NS was added, vortexed and 10ml of chloroform/methanol (2:1) was added, vortexed and left standing for 10 min, then 50ml (1mg/ml) of 1,2 – diacyl-sn-glycero-3-phosphocholine C17:0 (Lecithin C17:0 = LC17:0, C17:0 = 35) 492 μg, Avanti Polar Lipids Inc., Alabaster, CA) as an internal standard and 3ml of NS were added, vortexed and later centrifuged at 1000g for 15 min. The bottom layer was collected and evaporated to dryness under nitrogen. The methylation was executed by the BF3 in methanol (Sigma) at 90°C for 30 min. The fatty acid esters were extracted by hexane and ran on a gas chromatograph (Varian 3400 GC) with a 30-meter capillary column (DB-23, 0.25mm I.D., 0.25 mm coating thickness) and a flame ionization detector (FID). The helium carrier flow rate was 1.37ml/min (50°C). The airflow rate was 300ml/min. The nitrogen make up gas was 30ml/min. The hydrogen for FID was 30ml/min. The initial oven temperature was 50°C, holding 1 minute after injection. Then the temperature was programmed from 50°C to 180°C at a rate of 20°C/min, then from 180°C to 210°C and held for 3 min, then from 210°C at a rate of 20°C/min and held at the final temperature for 1 min. The injector and detector temperature were 240°C and 250°C, respectively. The Auto Sampler 8100 (Varian) was programmed to rinse twice with hexane before and after injection, and then inject 1ml of sample with a splitless mode, slow injection rate (0.3ml/sec), 0.2 min of hot needle time, 0.6 min of purge activation time. The fatty acid methyl esters (from C8:0 to C24:1 n-9) were identified by comparison of retention times with authentic standards (GLC-411; C17:0 from Nu-Check-Prep, EPA from Sigma) and the peak areas were integrated and reported using a Varian Star Workstation Computing Integrator and stored in the hard disk of a 386 computer. The standard was ran two times before and after running the sample group for the quality control of the GC condition.

Results

Four normal and seven cancer cell lines were analyzed for total fatty acids from C8:0 to C24:1 n-9. FCS was high in C18:2 n-6 (LA), C20:0, C20:2 n-6 and low in AA and DHA. The quantity of LA (C18:2 n-6) was 383 11 ± 6.26 mg/ml. We used 10% FCS in our culture media, which the final FCS concentration was 38.3μm/ml.

Our results were as follows: PA, OA, LA, EPA, SFA/ PUFA, total MFA and total (n-6) / (n-3) were lower in all the normal cell lines, while SA, DGLA, AA, DHA, PUFA, total n-3, total n-6 and SA? OA ratios were higher in normal cell lines than in their malignant counterparts. Marked differences between and among colon and lung cancer lines (normal and malignant) were found. For example, LA was lower in the normal colon cell line (CCD-18Co) than in the colon cancer cell line (LS-180). A similar trend was found in normal lung cell line (CCD-37lu) and lung malignant cell line (SK-MES-1).

Various fatty acids composition comparisons seem consistent in normal and malignant colon, such as in lung normal and malignant cell lines. Half the correlations (elongation and desaturation) resulted in a significant difference between normal and malignant cell lines.

In the colon cell lines, LA was lower in the normal cell line than in the malignant variant. While in contrast AA was higher in the normal cell line when compared to the malignant counterpart. EPA was higher, while DHA was lower in the malignant cell lines as compared to the normal cell line. Similar results were obtained for the lung cell lines.

In relation to the ratio of SA/OA of total lipids of the 11 tested cell lines. All normal cell lines resulted over 0.7 while the malignant cell lines were less than 0.7. All normal cell lines were significantly different from all cancer lines respectively ($p<0.01$).

In reference to the relationship of LA and AA percentages of the cell lines, the cancer cell lines had less LA, and more AA ($n=68, r=-0.95, p<0.001$). The coefficients of variances CV were very high for LA and AA (72.5% and 64.1%, respectively). A relatively weak correlation for LA and AA ($n=29, r=-0.58, p<0.005$) with a CV of 7.9 and 14.3 respectively for normal cell lines. These findings may reflect the varying abilities to incorporate LA and AA by the different cell lines.

Discussion

Fatty acids in a cell may serve as an energy source, structural components of biological membranes and precursors of the prostaglandin cascade, all which can deeply affect cellular metabolism and reproduction. It is conceivable that changes in fatty acids may be determinant of cellular metabolism including cell reproductive behavior. Our study shows a consistent regularity in fatty acid composition among normal cell lines a trend also repeated among the malignant cell lines, but different between normal and malignant.

Saturated fatty acids. Saturated fatty acids are synthesized from acetyl-CoA and malonyl-CoA to palmitic acid and then elongated to longer chain fatty acids. In normal cells, the correlation of the elongation from PA is positive. The more C16:0, the more C18:0, C20:0, C22:0 and C24:0 ($r = 0.511$). However, this correlation is quite contrary in malignant cell lines ($r = 0.608$).

The longer chain SFAs when incorporated into biological membranes decreases the fluidity of the membranes, hence decreasing the material exchange. So the higher metabolism of cancer cells might benefit from a lower incorporation of SFAs. On the other hand, higher percentages of C16:0, C18:1, n-9 and lower C18:0 in malignant cells might reflect their active desaturation from C18:0 to C18:1 n-9.

Monounsaturated fatty acids. It has been reported that the ratios of SA/OA were lower in blood of cancer patients than in people without diagnosed malignancy (13-15). This ratio has been suggested as a possible solid tumor marker (13, 16-18). The data presented here is in accordance to these previous reports. In the normal cell lines the SA/OA ratio was above 0.7 while in the malignant cell lines, the ratio was below 0.7 ($p<0.05$).

Polyunsaturated fatty acids. Total PUFA in malignant cell lines were lower than that of normal lines ($p<0.01$). The total PUFA was mainly composed of C18:2 n-6 and C20:4 n-6. The lower PUFA content was probably due to a decrease in C20:4 n-6 resulting from a lower 6Ä desaturase enzyme activity (19) Nevertheless C20:2 n-6 was higher in cancer cell lines ($p<0.001$) probably due to an active elongase enzyme activity. We should mention that malignant cell lines C-4I, Caov-3 and PC-3 did not show a marked decrease in both elongation and desaturation mechanisms.

Since our data shows differences in fatty acid composition between normal and malignant cell lines, it can be suggested that certain fatty acids may have a cytotoxic or cytostatic effect on particular cell lines; while others seem to enhance cell proliferation. In-vivo studies suggest Omega 3: EPA and DHA – as probably the more cytotoxic or cytostatic fatty acids to malignant cells (4,5). Since changes in fatty acid composition are attainable by dietary means, manipulation of membrane fatty acids may be a very viable adjuvant therapy against solid malignant tumors.

Resumen

Se midieron veintiocho ácidos grasos (C8:0 a C24:1 n-9) por cromatografía de gas en cuatro líneas celulares normales (C3H / 10T1 / 2, CCD-18Co, CCD-25SK y CCD-37Lu) y siete líneas celulares malignas (C-41, Caov-3, LS-180, PC-3, SK-MEL-28, SK-MES-1 y U-87 MG). Al compararse, se encontraron diferencias en contenido y proporciones de ácidos grasos. Las líneas celulares malignas demostraron menor contenido de C20: 4 n-6, C24:1 n-9, ácidos grasos poli-insaturados (PUFA's) y menor proporciones de C20:4 n-6 a C20:5 n-3, C16:0 a C18:1 n-9 y de esteárico a oleico (SA/OA) que su contraparte normal. Todas las líneas celulares malignas tenían una proporción de SA / OA menor de 7.0, mientras las lineas celulares normales tenían proporciones mayores 0.7 ($p<0.05$). Además la proporción de ácidos grasos saturados (SFA) a PUFA'S y la concentración de C18:1 n-9, C18:2 n-6, C20:5 n-3 fueron mayores en las líneas celulares normales. Se detectó una correlación positiva entre C16:0 y SFA'S de mayor longitud ($r=+0.511, p<0.05$) en las lineas celulares normales; sin embargo se encontró una correlación negativa ($r=0.608, p<0.05$) para líneas celulares malignas. Además, las líneas celulares malignas exhibieron a un

defecto particular de desaturación y una anormalidad en la incorporación de los ácido grasos C18:2 n-6 y C20-4 n-6.

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