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Changes in electric charge and phospholipids composition in human colorectal cancer cells

Izabela Dobrzyńska,[1] Barbara Szachowicz-Petelska,[1] Stanisław Sulkowski[2] and Zbigniew Figaszewski[1,3]

[1]*Institute of Chemistry, University in Białystok, Al. Piłsudskiego 11/4, Białystok, Poland;* [2]*Department of Pathology, Medical University of Białystok, Waszyngtona 13, Białystok, Poland;* [3]*Laboratory of Electrochemical Power Sources, Faculty of Chemistry, University of Warsaw, Warsaw, Poland*

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Abstract

Cancer cells perform their malicious activities through own cell membranes that screen and transmit inhibitory and stimulatory signals out of the cells and into them. This work is focused on changes of phospholipids content (PI – phosphatidylinositol, PS – phosphatidylserine, PE – phosphatidylethanolamine, PC – phosphatidylcholine) and electric charge that occur in cell membranes of colorectal cancer of pT3 stage, various grades (G2, G3) and without/with metastasis. Qualitative and quantitative composition of phospholipids in the membrane was determined by HPLC (high-performance liquid chromatography). The surface charge density of colorectal cancer cell membranes was measured using electrophoresis. The measurements were carried out at various pH of solution. It was shown that the process of cancer transformation was accompanied by an increase in total amount of phospholipids as well as an increase in total positive charge at low pH and total negative charge at high pH. A malignant neoplasm cells with metastases are characterized by a higher PC/PE ratio than malignant neoplasm cells without metastases. (Mol Cell Biochem **276:** 113–119, 2005)

Key words: phospholipids, electric charge, colorectal cancer cells

Introduction

Physiologic cell function are conditioned by intracellular environment. The effective barrier that surrounds and protects intracellular content, is a plasmalemma, that is employed in lots of cellular changes [1]. Cancer cells perform their malicious activities through their own cell membranes that screen and transmit inhibitory and stimulatory signals out of the cells and into them. Neoplasms produce and secrete agents that are found only in trace portions inside of cells. They are cancer markers that include carcinogenic antigens, hormones, metabolites, growth factors, enzymes and cytokines [2–5]. These agents are transported through cell membrane. In malignant cells the ultrastructural architecture of plasmalemma changes what results in alterations of biologic properties. Quantities of membrane components vary on a wide scale. Mostly protein and lipid content are affected due to disorders enzymes that take part in biosyntesis. Quantitative alterations of phospholipids and structural proteins are presumed to reflect disintegration and deep impairment of genomic functioning due to mutations as hallmarks of malignant transformation [6, 7]. A product of gene expression is undoubtedly engaged in maintaining of normal development of processes in healthy cells. Mutation, in some cases, might be connected with the virus. At an early stage of their oncogenic action virions attach to suitable membrane receptors of the host. This event can be prevented by immunoglobins cell surface in cooperation with lipids and structural proteins of plasmalemma. Carbohydrate residues connect with mentioned components of membrane and are situated on cell surface in hydrophilic

Address for offprints: Z.A. Figaszewski, Institute of Chemistry, University of Białystok, Al. Piłsudskiego 11/4 Białystok, Poland (E-mail: elchem@uwb.edu.pl)

phase. In this way carbohydrate residues create asymmetry of cell membrane, what is associated with superficial layer that covers the external surfaces of plasmalemmas [8]. This layer develops electric potential of the cell surface. A value of electric charge that is membrane accumulated on cell surface, reflects condition of the cell. A progress of malignancies often is associated with an increase of superficial membrane potential. In opposition, a decrease of the potential is detected in case of cell death. An electric potential in caused by carriers of negative electric charge that include phosphatidylserine, sialic acid, glicoforins, free carboxyl groups of peptides and molecules that contain plus charged amine groups of protein and aminophospholipids. A constant support of appropriate potential is required for normal development of metabolic processes that involve a role of cell membrane. Electric features of membrane result from an acid-base equilibrium and sophisticated interactions between membrane components and surrounding elements. The equilibrium is set up by most of membrane molecules like phospholipids and proteins.

The work is focused on changes of phospholipids content and electric charge that occur in cell membranes of colorectal cancer.

Materials and methods

Tissue samples were obtained from 18 patients (9 men and 9 women) who underwent surgical resection because of colorectal cancer. Our study included only colorectal cancers classified histopathologically as adenocarcinoma: 13 cases in G2 grade and 5 cases in G3 grade. There were all tumors in pT3 stage. 10/18 (55.6%) patients had involved lymph (N+) nodes at the time of diagnosis. The age of patients ranged from 38 to 83 years old. Tumor samples with adjacent normal colon mucosa were collected immediately after tumor removal.

Isolation and analysis of phospholipids by HPLC method

The tissues were homogenated in 1 mM (pH = 7.6)-0.5 M $CaCl_2$ until almost all of cells and approximately 90% their nuclei were lysed. Membrane fragments, undisrupted cells, nuclei and elements of connective tissue were spinned. The sedimentation was washed and partially separated in two following spinning at $1000 \times g$. The sedimentary deposit was homogenated in saccharose of 1.22 density and in the next step was covered with saccharose of 1.16 density. The cell membranes were separated by centrifugation at $2000 \times g$ for 25–35 min [9]. Then a method of Folch was applied to extraction of phospholipids [10]. Tissue was homogenized in a chloroform–methanol mixture of 2:1 volume ratio using 20 cm^3 per g of tissue. The solution was then filtered out with degreased paper filters, the precipitate was washed with the extracting solution containing 0.05 M calcium chloride; the volume ratio of chloroform, methanol and aqueous calcium chloride solution was 8:4:3. The suspensions was centrifuged at $500 \times g$ for 2 min, the organic and the aqueous phases were separated, the aqueous phase was shaken again with chloroform, methanol and water mixture of 3:48:47 volume ratio and the phases were separated. The organic phases were combined and were evaporated to dryness. The extract were dissolved in 200 μl of heksane–isopropanol mixture (3:2) [10].

The HPLC analysis was then carried out. The isolated phospholipids were separated by group analysis in silica gel column using NP-HPLC (liquid chromatography in normal phase system); acetonitryle–methanol–85% phosphoric acid mixture in 130:5:1.5 volume ratio in isocratic elution at 1 ml/s flow rate and 214 nm wave lenght [11].

Electrochemical method

In order to determine surface charge density of cell membrane, colorectal cancer tissue from human was exposed to trypsin action. Received cells were put into the measuring vessel, then electrophoretic mobility was measured by using DTS5300 ZETASIZER 3000 apparatus (MALVERN INSTRUMENTS).

The surface charge density has been determined using equation: $\sigma = \eta u/d$; here u is electrophoretic mobility, η is viscosity of solution, d is diffuse layer thickness [12].

The diffuse layer thickness was determined from the formula [13] $d = \sqrt{\frac{\varepsilon \cdot \varepsilon_0 \cdot R \cdot T}{2 \cdot F^2 \cdot I}}$, where R is the gas constant, T is the temperature, F is the Faraday number and I is the ionic strength of 0.9% NaCl, $\varepsilon\varepsilon_0$ – are relative and absolute permittivities of the medium.

Statistical methods

The data obtained in this study are expressed as mean $\pm$ SD. The data were analysed using Wilcoxon Matched-Pairs Signed-Ranks Test (from standard statistical program SPSS 8.0 PL) for comparisons to determine significance between control and cancer. The values for $p < 0.05$ were considered significant.

Results

Content of phospholipids, their surface concentration and surface fraction occupied by them as well as the surface concentration of acidic groups (C_{TA}) and the surface concentration of basic groups (C_{TB}) parameters of human large intestine cell membrane unmodified and of the membrane modified by neoplasm lesion are presented in Tables 1–3. Generally,

Table 1. Content of phospholipids and C_{TA} i C_{TB} of human large intestine of pT3 stage, G2 grade without metastasis (N−)

No. of patients	Phospholipids	HPLC method						Electrophoretic method			
		Content of phospholipid of plasmalemma (mg/g tissue)		Surface concentration of phospholipids (10^{-7} mol/m^2)		Fraction of surface of occupied by phospholipids		C_{TA} (10^{-7} mol/m^2)		C_{TB} (10^{-7} mol/m^2)	
		Control	Tumour	Control	Tumour	Control	Tumour	Control	Tumour	Control	Tumour
1	PI	0.010 ± 0.002	0.225 ± 0.020[a]	0.042 ± 0.002	0.996 ± 0.002[a]	0.003 ± 0.001	0.063 ± 0.002[a]	2.71 ± 0.10	3.73 ± 0.12[a]	0.90 ± 0.05	1.35 ± 0.08[a]
	PS	0.016 ± 0.003	0.100 ± 0.010[a]	0.076 ± 0.007	0.465 ± 0.020[a]	0.003 ± 0.001	0.020 ± 0.001[a]				
	PE	0.550 ± 0.010	0.890 ± 0.030[a]	2.445 ± 0.120	3.950 ± 0.160[a]	0.060 ± 0.001	0.101 ± 0.099[a]				
	PC	0.675 ± 0.011	1.100 ± 0.061[a]	3.181 ± 0.254	5.170 ± 0.361[a]	0.163 ± 0.010	0.265 ± 0.022[a]				
2	PI	0.012 ± 0.003	0.239 ± 0.040[a]	0.051 ± 0.003	1.018 ± 0.080[a]	0.003 ± 0.001	0.065 ± 0.003[a]	1.92 ± 0.12	3.25 ± 0.12[a]	0.63 ± 0.04	1.24 ± 0.06[a]
	PS	0.028 ± 0.002	0.151 ± 0.022[a]	0.132 ± 0.015	0.710 ± 0.044[a]	0.005 ± 0.001	0.030 ± 0.001[a]				
	PE	0.510 ± 0.020	0.740 ± 0.081[a]	2.260 ± 0.208	3.290 ± 0.160[a]	0.060 ± 0.001	0.080 ± 0.009[a]				
	PC	0.116 ± 0.010	1.237 ± 0.099[a]	2.870 ± 0.228	4.400 ± 0.234[a]	0.150 ± 0.020	0.225 ± 0.013[a]				
3	PI	0.074 ± 0.008	0.081 ± 0.007	0.314 ± 0.010	0.348 ± 0.010[a]	0.020 ± 0.011	0.022 ± 0.012	2.41 ± 0.12	2.84 ± 0.09[a]	0.74 ± 0.07	0.97 ± 0.08[a]
	PS	0.086 ± 0.006	0.131 ± 0.010[a]	0.399 ± 0.018	0.620 ± 0.030[a]	0.016 ± 0.009	0.025 ± 0.018				
	PE	0.494 ± 0.021	0.902 ± 0.051[a]	2.200 ± 0.290	4.010 ± 0.201[a]	0.060 ± 0.021	0.103 ± 0.020[a]				
	PC	0.648 ± 0.024	1.240 ± 0.085[a]	3.040 ± 0.330	5.810 ± 0.385[a]	0.156 ± 0.021	0.297 ± 0.026[a]				
4	PI	0.087 ± 0.009	0.248 ± 0.020[a]	0.368 ± 0.015	1.056 ± 0.065[a]	0.023 ± 0.012	0.067 ± 0.012[a]	2.08 ± 0.09	2.45 ± 0.11[a]	0.67 ± 0.04	1.15 ± 0.06[a]
	PS	0.097 ± 0.007	0.097 ± 0.006	0.451 ± 0.022	0.450 ± 0.022	0.019 ± 0.010	0.018 ± 0.001				
	PE	0.901 ± 0.050	0.932 ± 0.050	4.000 ± 0.250	4.130 ± 0.230	0.102 ± 0.018	0.106 ± 0.019				
	PC	1.139 ± 0.061	1.245 ± 0.089[a]	5.347 ± 0.320	5.834 ± 0.310	0.274 ± 0.026	0.299 ± 0.021				
5	PI	0.064 ± 0.005	0.109 ± 0.010[a]	0.280 ± 0.020	0.468 ± 0.020[a]	0.018 ± 0.001	0.030 ± 0.009[a]	2.24 ± 0.11	2.91 ± 0.22[a]	0.91 ± 0.05	1.39 ± 0.06[a]
	PS	0.086 ± 0.004	0.114 ± 0.015[a]	0.415 ± 0.030	0.529 ± 0.031[a]	0.017 ± 0.002	0.022 ± 0.008				
	PE	0.498 ± 0.012	0.768 ± 0.080[a]	2.210 ± 0.160	3.410 ± 0.124[a]	0.060 ± 0.020	0.090 ± 0.019				
	PC	0.677 ± 0.018	1.054 ± 0.095[a]	3.170 ± 0.200	4.940 ± 0.281[a]	0.162 ± 0.019	0.253 ± 0.022[a]				
6	PI	0.020 ± 0.002	0.056 ± 0.006[a]	0.085 ± 0.012	0.238 ± 0.012[a]	0.005 ± 0.001	0.015 ± 0.001[a]	2.19 ± 0.08	2.81 ± 0.14[a]	0.69 ± 0.05	1.65 ± 0.06[a]
	PS	0.024 ± 0.002	0.096 ± 0.009[a]	0.112 ± 0.018	0.310 ± 0.018[a]	0.005 ± 0.001	0.013 ± 0.001[a]				
	PE	0.432 ± 0.012	0.951 ± 0.092[a]	1.920 ± 0.201	4.220 ± 0.180[a]	0.050 ± 0.009	0.108 ± 0.020[a]				
	PC	0.707 ± 0.019	1.368 ± 0.101[a]	3.320 ± 0.202	6.420 ± 0.202[a]	0.169 ± 0.012	0.329 ± 0.025[a]				
7	PI	0.009 ± 0.001	0.030 ± 0.015[a]	0.035 ± 0.016	0.132 ± 0.021[a]	0.002 ± 0.001	0.008 ± 0.001[a]	1.56 ± 0.14	1.69 ± 0.12	0.66 ± 0.04	0.77 ± 0.04[a]
	PS	0.010 ± 0.011	0.021 ± 0.010	0.049 ± 0.018	0.098 ± 0.030[a]	0.002 ± 0.001	0.004 ± 0.001				
	PE	0.419 ± 0.023	0.828 ± 0.052[a]	1.860 ± 0.099	3.680 ± 0.160[a]	0.050 ± 0.015	0.090 ± 0.017[a]				
	PC	0.675 ± 0.034	1.182 ± 0.065[a]	3.160 ± 0.150	5.540 ± 0.390[a]	0.162 ± 0.016	0.284 ± 0.019[a]				
8	PI	0.036 ± 0.012	0.136 ± 0.016[a]	0.153 ± 0.018	0.581 ± 0.081[a]	0.010 ± 0.013	0.037 ± 0.003[a]	2.45 ± 0.12	2.66 ± 0.21	0.61 ± 0.03	0.72 ± 0.05[a]
	PS	0.042 ± 0.015	0.103 ± 0.050[a]	0.197 ± 0.060	0.488 ± 0.079[a]	0.008 ± 0.001	0.020 ± 0.009[a]				
	PE	0.468 ± 0.028	0.895 ± 0.039[a]	2.080 ± 0.101	3.980 ± 0.210[a]	0.050 ± 0.008	0.102 ± 0.012[a]				
	PC	0.686 ± 0.039	1.287 ± 0.070[a]	3.210 ± 0.155	6.030 ± 0.528[a]	0.164 ± 0.012	0.309 ± 0.018[a]				

PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; PC: phosphatidylcholine.
Statistically significant differences for $p < 0.05$.
[a]In comparison with control.

Table 2. Content of phospholipids and C_{TA} i C_{TB} of human large intestine of pT3 stage, G2 grade and with metastases (N+)

No. of patients	Phospholipids	HPLC method						Electrophoretic method			
		Content of phospholipid of plasmalemma (mg/g tissue)		Surface concentration of phospholipids (10^{-7} mol/m^2)		Fraction of surface of occupied by phospholipids		C_{TA} (10^{-7} mol/m^2)		C_{TB} (10^{-7} mol/m^2)	
		Control	Tumour	Control	Tumour	Control	Tumour	Control	Tumour	Control	Tumour
9	PI	0.044 ± 0.002	0.225 ± 0.011[a]	0.186 ± 0.009	0.961 ± 0.020[a]	0.012 ± 0.002	0.061 ± 0.003[a]	1.88 ± 0.10	2.23 ± 0.16[a]	0.70 ± 0.05	0.91 ± 0.08[a]
	PS	0.043 ± 0.002	0.145 ± 0.009[a]	0.201 ± 0.007	0.679 ± 0.015[a]	0.008 ± 0.001	0.028 ± 0.002[a]				
	PE	0.642 ± 0.011	0.909 ± 0.019[a]	2.850 ± 0.135	4.040 ± 0.301[a]	0.070 ± 0.002	0.103 ± 0.008[a]				
	PC	0.783 ± 0.012	1.406 ± 0.025[a]	3.680 ± 0.148	6.580 ± 0.399[a]	0.188 ± 0.008	0.337 ± 0.009[a]				
10	PI	0.150 ± 0.009	0.160 ± 0.008	0.633 ± 0.012	0.682 ± 0.016[a]	0.040 ± 0.005	0.043 ± 0.005	2.71 ± 0.12	4.94 ± 0.11[a]	0.77 ± 0.07	1.03 ± 0.08[a]
	PS	0.055 ± 0.003	0.055 ± 0.003	0.250 ± 0.009	0.250 ± 0.009	0.010 ± 0.003	0.010 ± 0.003				
	PE	0.540 ± 0.012	0.545 ± 0.013	2.390 ± 0.140	2.420 ± 0.108	0.060 ± 0.003	0.060 ± 0.003				
	PC	0.925 ± 0.022	1.555 ± 0.028[a]	4.341 ± 0.205	7.305 ± 0.505[a]	0.222 ± 0.009	0.374 ± 0.010[a]				
11	PI	0.044 ± 0.002	0.144 ± 0.009[a]	0.187 ± 0.011	0.607 ± 0.012[a]	0.012 ± 0.001	0.039 ± 0.004[a]	3.32 ± 0.11	4.35 ± 0.12[a]	1.17 ± 0.07	1.23 ± 0.07[a]
	PS	0.025 ± 0.001	0.075 ± 0.005[a]	0.107 ± 0.008	0.368 ± 0.010[a]	0.004 ± 0.001	0.015 ± 0.002[a]				
	PE	0.381 ± 0.018	0.396 ± 0.011	1.690 ± 0.084	1.760 ± 0.038	0.040 ± 0.005	0.040 ± 0.003				
	PC	0.475 ± 0.020	1.281 ± 0.016[a]	2.248 ± 0.135	6.020 ± 0.348[a]	0.115 ± 0.007	0.308 ± 0.009[a]				
12	PI	0.018 ± 0.001	0.113 ± 0.008[a]	0.077 ± 0.008	0.479 ± 0.010[a]	0.005 ± 0.001	0.030 ± 0.004[a]	2.90 ± 0.09	3.98 ± 0.08[a]	1.16 ± 0.09	1.68 ± 0.08[a]
	PS	0.031 ± 0.003	0.110 ± 0.007[a]	0.149 ± 0.013	0.520 ± 0.013[a]	0.006 ± 0.001	0.021 ± 0.003[a]				
	PE	0.551 ± 0.018	0.592 ± 0.013[a]	2.440 ± 0.149	2.630 ± 0.109[a]	0.060 ± 0.006	0.070 ± 0.006				
	PC	0.698 ± 0.021	0.933 ± 0.026[a]	3.270 ± 0.139	4.373 ± 0.658[a]	0.167 ± 0.015	0.224 ± 0.012[a]				
13	PI	0.026 ± 0.003	0.039 ± 0.003[a]	0.113 ± 0.007	0.166 ± 0.009[a]	0.007 ± 0.001	0.010 ± 0.002	2.66 ± 0.11	3.26 ± 0.14[a]	0.89 ± 0.05	1.57 ± 0.08[a]
	PS	0.013 ± 0.001	0.026 ± 0.002[a]	0.063 ± 0.002	0.123 ± 0.007[a]	0.002 ± 0.001	0.005 ± 0.001[a]				
	PE	0.433 ± 0.019	0.546 ± 0.014[a]	1.920 ± 0.011	2.420 ± 0.105[a]	0.050 ± 0.005	0.060 ± 0.005				
	PC	0.770 ± 0.030	1.240 ± 0.019[a]	3.600 ± 0.201	5.800 ± 0.565[a]	0.184 ± 0.022	0.297 ± 0.015[a]				

PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; PC: phosphatidylcholine.
Statistically significant differences for $p < 0.05$.
[a]In comparison with control.

Table 3. Content of phospholipids and C_{TA} i C_{TB} of human large intestine of pT3 stage, G3 grade and with metastasis (N+)

No. of patients	Phospholipids	HPLC method						Electrophoretic method			
		Content of phospholipid of plasmalemma (mg/g tissue)		Surface concentration of phospholipids (10^{-7} mol/m^2)		Fraction of surface of occupied by phospholipids		C_{TA} (10^{-7} mol/m^2)		C_{TB} (10^{-7} mol/m^2)	
		Control	Tumour	Control	Tumour	Control	Tumour	Control	Tumour	Control	Tumour
14	PI	0.200 ± 0.009	0.245 ± 0.010[a]	0.847 ± 0.030	1.000 ± 0.042[a]	0.054 ± 0.002	0.064 ± 0.003[a]	2.62 ± 0.08	4.77 ± 0.31[a]	1.07 ± 0.06	0.83 ± 0.09[a]
	PS	0.071 ± 0.005	0.100 ± 0.009[a]	0.335 ± 0.021	0.463 ± 0.040[a]	0.014 ± 0.002	0.019 ± 0.003				
	PE	0.462 ± 0.015	0.476 ± 0.019	2.050 ± 0.099	2.110 ± 0.101	0.050 ± 0.003	0.050 ± 0.003				
	PC	0.614 ± 0.018	1.476 ± 0.065[a]	2.880 ± 0.234	6.920 ± 0.308[a]	0.147 ± 0.020	0.354 ± 0.022[a]				
15	PI	0.046 ± 0.007	0.084 ± 0.008[a]	0.185 ± 0.015	0.379 ± 0.035[a]	0.012 ± 0.002	0.024 ± 0.004[a]	2.66 ± 0.14	3.38 ± 0.10[a]	0.90 ± 0.05	1.23 ± 0.09[a]
	PS	0.030 ± 0.006	0.034 ± 0.006	0.132 ± 0.011	0.169 ± 0.019[a]	0.005 ± 0.001	0.007 ± 0.001				
	PE	0.447 ± 0.016	0.456 ± 0.018	1.990 ± 0.096	2.020 ± 0.018	0.050 ± 0.013	0.050 ± 0.012				
	PC	0.677 ± 0.019	1.084 ± 0.050[a]	3.170 ± 0.301	5.080 ± 0.311[a]	0.162 ± 0.019	0.260 ± 0.024[a]				
16	PI	0.043 ± 0.006	0.078 ± 0.007[a]	0.178 ± 0.011	0.344 ± 0.030[a]	0.011 ± 0.002	0.022 ± 0.008[a]	1.86 ± 0.12	2.25 ± 0.12[a]	0.54 ± 0.06	0.62 ± 0.07
	PS	0.014 ± 0.002	0.057 ± 0.005[a]	0.066 ± 0.006	0.260 ± 0.018[a]	0.003 ± 0.001	0.011 ± 0.004[a]				
	PE	0.443 ± 0.017	0.478 ± 0.016[a]	1.970 ± 0.122	2.120 ± 0.012[a]	0.050 ± 0.012	0.050 ± 0.011				
	PC	0.707 ± 0.021	1.386 ± 0.065[a]	3.320 ± 0.205	6.500 ± 0.405[a]	0.170 ± 0.020	0.333 ± 0.040[a]				
17	PI	0.095 ± 0.008	0.245 ± 0.011[a]	0.414 ± 0.023	1.036 ± 0.055[a]	0.026 ± 0.005	0.066 ± 0.009[a]	2.88 ± 0.16	4.15 ± 0.33[a]	0.98 ± 0.09	1.42 ± 0.08[a]
	PS	0.115 ± 0.010	0.130 ± 0.010	0.550 ± 0.028	0.603 ± 0.042[a]	0.023 ± 0.004	0.025 ± 0.004[a]				
	PE	0.450 ± 0.019	0.620 ± 0.021[a]	2.000 ± 0.133	2.756 ± 0.119[a]	0.050 ± 0.006	0.070 ± 0.007[a]				
	PC	0.650 ± 0.020	0.701 ± 0.023[a]	3.046 ± 0.199	3.284 ± 0.201	0.156 ± 0.016	0.168 ± 0.020[a]				
18	PI	0.010 ± 0.002	0.810 ± 0.026[a]	0.046 ± 0.008	3.419 ± 0.202[a]	0.003 ± 0.001	0.218 ± 0.015[a]	2.03 ± 0.11	2.31 ± 0.09[a]	0.66 ± 0.06	0.75 ± 0.07
	PS	0.019 ± 0.002	0.022 ± 0.003	0.090 ± 0.009	0.104 ± 0.012	0.004 ± 0.001	0.004 ± 0.001				
	PE	0.417 ± 0.022	0.419 ± 0.019	1.850 ± 0.124	3.970 ± 0.320[a]	0.050 ± 0.006	0.102 ± 0.012[a]				
	PC	0.770 ± 0.044	1.197 ± 0.055[a]	3.610 ± 0.202	5.620 ± 0.455[a]	0.185 ± 0.018	0.288 ± 0.022[a]				

PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; PC: phosphatidylcholine.
Statistically significant differences for $p < 0.05$.
[a]In comparison with control.

an increase in the content of all phospholipids and in their surface concentration is observed in the patients at pT3 stage, G2 grade without metastasis (N−) with respect to the unaffected cells. However, phosphatidylcholine is most of all than other phospholipids, both in control tissue and in cancer tissues. An increase in acid and basic groups surface concentration was observed in the above mentioned patients with respect to unmodified cells. Only in patients 7 and 8 there were no statistically relevant C_{TA} changes.

The changes observed in patients at pT3 stage, G2 grade and with metastases (N+) proved to be similar to the patients without metastasis. A not significant increase in the phosphatidyl ethanol amine content and significant increase in the phosphatidylcholine content caused by cancer transformation was observed in the cell membrane (particularly in patients 10–12). Similarly, the C_{TA} value of the cell membranes modified by cancer transformation was higher than that of the unmodified cells.

The changes observed in patients at pT3 stage, G3 grade and with metastasis (N+) proved to be similar.

Discussion

A cancer transformation results in the appearance of a new cell line in the organism whose malignant activity to the organism is transmitted from one cell generation to another. It seems that phenomena related to cell membrane modification might play a role during the transformation of a normal cell into a cancer one. It is hard to decide if this modification is a cause or rather a result of cancer changes. The results of our research show changes in the composition of membrane in all examined cases. In order to explain this problem we should analyse changes in cell membrane in premalignant cases of colon cancer (directly preceding the growth of cancer for example adenomas). These transformations also result in serious metabolic track perturbations; the perturbations are reflected by changes in the content of phospholipids and proteins in biological membranes [14]. The phospholipids are an integral part of the membrane and determine its structure. In the most colorectal cancer results in increased amount of all phospholipids of cell membrane (Tables 1–3). The literature data show that an increase in the amount of phospholipids can generally be observed in human colon cancer [15] and in murine tumour mammary [16]. It has been suggested that higher amount of phospholipids can be due to enhanced cell membrane synthesis related to accelerated neoplasm cell replication [3]. The mechanisms which are responsible for an increase in the amount of phospholipids can vary depending on cell nature, cell growth phase and its malignancy. The greatest changes in the content of phosphatidylcholine and phosphatidylethanolamine were observed in the first phase G_1 of cell cycle, in which activity of the enzymes controlling biosynthesis, catabolism and metabolism of phospholipids attains maximum [17, 18]. The characteristic parameters of phospholipids presented in Tables 1–3 also show that the content of phosphatidylcholine in normal mucosa or lesions of colorectal cancer cells is higher than that of other phospholipids. Its amount is higher in cancer cells. Earlier reports [15] are confirmed by these observations.

Differences in membrane phospholipid contents can affect the tendency to produce metastasis. A malignant neoplasm cells with high number of metastases are characterized by a higher phosphatidylcholine/phosphatidylethanolamine ratio than malignant neoplasm cells with low number of metastases [19]; it has been confirmed by the results of our work.

Increased amount of phospholipids results in a higher amount of functional groups: amino, carboxy and phosphate groups. In acid medium (low pH), the charge of phospholipids is mainly due to amino groups whereas in basic medium (high pH) it is due to carboxy and phosphate groups. Increased amount of phospholipids can increase surface density of negatively charged groups of large intestine cell membrane at low pH values and that of positively charged ones at high pH; it has been confirmed by the results of this work (Tables 1–3). The main component of the large intestine cell membrane outer layer is phosphatidylcholine and its higher content can provoke an increase in both positively charged groups concentration at low pH values and in negatively charged groups concentration at high pH values.

The literature data show that in tumors both hypoxia and acidity could exist. Hypoxia is an important cellular stressor that triggers a survival program by which cells attempt to adapt to the new environment. This primarily involves adaptation of methabolism and/or stimulation of oxygen delivery [20]. Hypoxia/reoxygenation and acidity induced exposure of anionic phospholipid, most likely phosphatidylserine and phosphatidylethanolamine [21, 22]. Anionic phospholipids on tumor vessels could potentially provide markers for tumor vessel targeting and imaging [22]. It seems that the alterations in the distribution of phosphatidylserine, which is a component of the skeleton, could cause an increase in negatively charged groups concentration at high pH values.

Beside the phospholipids which have been discussed in this work, the cell membrane charge is also affected by sialic acid being the component of glycolipids and glycoproteins. It has been supposed that sialic acid also influences surface concentration of acid and basic groups as well as association constants of positive and negative groups during cancer transformation. Increased sialic acid content in glycolipids and glycoproteins has been confirmed by literature data [23, 24]. Increased sialic acid content can provoke increased surface concentration of acid groups.

Electrophoretically determined functional group surface concentrations in human large intestine cell membrane is of the order of 10^{-7} mol/m^2 (Tables 1–3) whereas functional group surface concentration calculated from the amount of all four phospholipids as determined by the HPLC method is higher. As the functional groups of proteins are manifested in the electrophoretic studies only, the lower electrophoretic study result can be due to screening of a part of phospholipids by glycoproteins situated at the membrane surface. Therefore, the cell membrane contains more functional groups originating from phospholipids.

To sum up, the cell membrane structure and the function are modified by neoplasm lesion. It is reflected by changes in the amount of phospholipids and in the electric charge of human thick intestine cell membrane.

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