# Structure and Properties of Lipid Bilayer of Erythrocyte Membranes in Patients with Malignant Tumors

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The study of erythrocyte membranes in untreated patients with malignant tumors of different location (lung cancer, tumors of the head and neck, stomach and colorectal cancer) revealed changes in the fatty-acid spectrum of phospholipid fraction paralleled by an increase in the viscosity of the lipid bilayer, including the area of protein-lipid contacts. The degree of changes depended on tumor location.

**Key Words:** *erythrocyte; membrane; fatty acids; microviscosity; patients with malignant tumors*

Despite the appearance of numerous reports about the structure of membranes and metabolism of mature red blood cells during tumor growth, some aspects of this problem remain little studied [5,13,14]. The important role of erythrocyte membranes in their functions and rapid reaction of erythrocytes (changes in their morphofunctional characteristics) to toxic factors even of low intensity suggest that study of the membrane status of mature red blood cells in patients with malignant tumors can provide valuable information on functional intactness of erythrocytes, which is particularly important during the period preceding the manifestation of changes in quantitative parameters of the erythron [6,7]. Hence, we studied the structure and characteristics of the lipid bilayer of erythrocyte membranes in patients with lung cancer, tumors of the head and neck, stomach and colorectal cancer by gas-liquid chromatography and fluorescent probing.

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## MATERIALS AND METHODS

Fifty patients with tumors of different location (36 men and 14 women aged 43-67 years) were examined. This group included patients with stages III-IV tumors of the following locations: lung cancer ($n$=13), tumors of the head and neck ($n$=12), stomach cancer ($n$=16), colorectal cancer ($n$=9). The diagnosis in all cases was based on clinical data and findings of instrumental methods of examination (endoscopy, X-ray examination, *etc.*) with obligatory morphological confirmation. Hematological tests were carried out before treatment. Control group consisted of 30 healthy volunteers (18 men and 12 women) aged 21-48 years. Differences in the studied parameters between males and females (both cancer patients and healthy volunteers) were statistically negligible. Venous blood was analyzed.

Erythrocyte membranes were isolated by the method of J. T. Dodge *et al.* [11]. Fatty-acid composition of erythrocyte membrane phospholipids was analyzed by gas-liquid chromatography [3]. Separation of fatty acids was carried out on a Tracor-540 chromatographer. The peaks were identified using fatty acid esters and their standard mixtures (Sigma). Intrinsic fluor-

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escence of erythrocyte ghosts and fluorescence of the probe in the erythrocyte membrane was measured on a Hitachi-MPF-4 spectrofluorometer. Pyrene (Sigma) served as a fluorophore. All molecules of the probe were excited at an excitation wavelength 340 nm, but only pyrene molecules in the protein-lipid contact area were excited at $\lambda_{ex}$=285 nm. Two peaks (370 and 390 nm) in the spectrum of pyrene fluorescence in erythrocyte membranes were caused by fluorescence of pyrene monomer, while a 470-nm peak reflected fluorescence of pyrene eximer (dimer). The ratios of fluorescence intensities $J_{370}/J_{470}$ and $J_{390}/J_{470}$ characterize microviscosity, while the $J_{370}/J_{390}$ ratio reflects polarity of probe microenvironment. The *R* value (percentage of energy migration) corresponds to the percentage of energy transfer from tryptophan molecules to pyrene [1]. The significance of intergroup differences was evaluated using Student's *t* test for cases with normal distribution and using nonparametrical tests (Mann—Whitney's *U* test and van der Warden test) for non-normal distributions.

## RESULTS

The growth of malignant tumors is paralleled by disorders in lipid metabolism in tumor tissue and in the whole body, including blood cells [6,9]. Our previous study of the erythrocyte membrane lipid spectrum in tumor patients [6] revealed a significant decrease in the content (g/liter) of total lipids (in lung cancer, head and neck tumors, stomach and colorectal cancer), total phospholipids (head and neck tumors, stomach and colorectal cancer), and absolute content of phosphatidylcholine and phosphatidylethanolamine fractions in erythrocyte membranes in patients with stomach cancer and head and neck tumors. The absolute content of phosphatidylcholine and phosphatidylethanolamine fractions in erythrocyte membranes of patients with lung cancer and colorectal cancer virtually did not differ from that in normal subjects.

Physicochemical characteristics of lipids, in particular, phospholipids, are largely determined by their fatty acid constituents [2,4,12], which prompted us to study the state lipid bilayer in erythrocyte membranes. We revealed redistribution of the main fatty acids (palmitic, stearic, oleic, linoleic, and arachidonic) in phosphatidylcholine and phosphatidylethanolamine fractions of erythrocyte membranes in cancer patients (Table 1). In patients with lung cancer, stomach cancer, and colorectal cancer the content of arachidonic acid in the phosphatidylcholine fraction of erythrocyte membranes significantly decreased; this decrease was most pronounced in patients with colorectal cancer in comparison with normal subjects (Table 1). Moreover, the content of linoleic acid in the phosphatidylcholine fraction of erythrocyte membranes significantly decreased in patients with lung cancer compared to donors. No appreciable changes in the content of the studied fatty acids were detected in this phospholipid fraction of patients with tumors of the head and neck. As for phosphatidylethanolamine fraction of erythrocyte membranes, the decrease in the content of arachi-

**TABLE 1.** Fatty Acid Content in Phosphatidylcholine and Phosphatidylethanolamine Fractions of Erythrocyte Membranes of Patients with Malignant Tumors of Different Location (*X*±*m*, %)

| Acid | Donors (control) | Patients | | | |
|---|---|---|---|---|---|
| | | lung cancer | head and neck tumors | stomach cancer | colorectal cancer |
| Phosphatidylcholine fraction | | | | | |
| palmitic | 37.34±3.36 | 38.20±4.29 | 39.33±4.76 | 31.94±6.00 | 36.97±3.06 |
| stearic | 26.77±3.42 | 30.76±5.52 | 28.51±3.76 | 34.28±3.21 | 24.82±2.40$^{o}$ |
| oleic | 21.82±3.80 | 25.66±4.13 | 22.53±2.37 | 26.12±5.97 | 29.19±3.03 |
| linoleic | 7.87±1.29 | 3.18±0.99** | 6.07±0.76$^{+}$ | 4.94±1.25 | 7.50±1.50$^{+}$ |
| arachidonic | 6.20±1.26 | 2.20±0.63** | 3.56±1.12 | 2.72±0.83*** | 1.52±0.52* |
| Phosphatidylethanolamine fraction | | | | | |
| palmitic | 34.76±3.11 | 29.07±4.21 | 39.86±6.96 | 32.22±4.74 | 33.47±3.81 |
| stearic | 22.64±1.38 | 31.50±2.16* | 28.06±4.62 | 37.20±3.45* | 26.07±2.24$^{o}$ |
| oleic | 28.19±5.16 | 28.51±3.88 | 24.83±4.45 | 23.32±3.65 | 31.32±2.02 |
| linoleic | 5.69±1.09 | 8.31±2.75 | 4.28±0.80 | 5.70±1.41 | 7.25±1.95 |
| arachidonic | 8.72±1.36 | 2.61±0.64* | 2.97±1.80*** | 1.56±0.31* | 1.89±0.77* |

**Note.** *$p$<0.01, **$p$<0.02, ***$p$<0.05 compared to donors (control); $^{+}p$<0.05 compared to patients with lung cancer; $^{o}p$<0.05 compared to patients with stomach cancer.

donic acid in all groups of cancer patients was paralleled by an increase of stearic acid content in patients with lung and stomach cancer in comparison with healthy subjects (Table 1). Hence, despite the absence of significant differences in the absolute content of phosphatidylcholine and phosphatidylethanolamine in erythrocyte membranes of patients with lung cancer and colorectal cancer in comparison with the control [6], we detected significant changes in the content of the main fatty acids in these phospholipid fractions.

In addition, we found that the ratio of arachidonic to linoleic acid ($C_{20:4}/C_{18:2}$) in the phosphatidylethanolamine fraction of erythrocyte membranes in patients with tumors of the head and neck, stomach and colorectal cancer was lower than in normal controls; this ratio also decreased in the phosphatidylcholine fraction of patients with colorectal cancer (Table 2). Presumably, the detected decrease in arachidonic acid content was due to disturbances in its formation from linoleic acid [4]. The ratio of the sum of saturated to polyunsaturated fatty acids in the phosphatidylcholine fraction of erythrocyte membranes significantly increased in patients with lung cancer, stomach cancer, and head and neck tumors in comparison with healthy subjects. Similar changes were observed in the phosphatidylethanolamine fraction of erythrocyte membranes in patients with stomach cancer and head and neck tumors (Table 2).

It is known that lipid components (mainly phospholipids and cholesterol) determine microviscosity of membranes. Microviscosity of the membrane lipid bilayer depends on the length of the phospholipid acyl chains, degree of their unsaturation, and concentration of bivalent cations in the medium. Changes in unsaturation of phospholipid acyl chains modify physical properties of biomembranes (permeability, deformability, *etc.*) [2,4]. For evaluation of the state of erythrocyte membranes we used fluorescent probe pyrene (Table 3). The study of fluorescence parameters of the lipotropic probe pyrene showed that tumor growth was associated with pronounced disorders in the membrane status of mature circulating erythrocytes (increased viscosity of membrane lipid phase in general, including areas of the protein-lipid contact; increased polarity of pyrene microenvironment). Increase in fluorescence parameters of pyrene probe, characterizing disorders in the structure and function of the deep layers of erythrocyte membranes, and increase in their microviscosity can be caused by essential changes in the composition of the erythrocyte membrane lipid bilayer, which can result from intensification of LPO processes [1,8,10]. Changes in the lipid composition of erythrocyte membranes, including a decrease in the level of fatty acids (lipid components) unsaturation, were also detected in our study.

Changes in the lipid bilayer of the erythrocyte membrane during tumor process can modulate ion transport, membrane permeability for various compounds, lateral diffusion of receptors, and enzyme activity, which lead to dysfunction of red blood cells and, eventually, to augmentation of the disease. Universal changes in the erythrocyte membrane bilayer

**TABLE 2.** Fatty-Acid Spectrum of Phosphatidylcholine and Phosphatidylethanolamine Fractions of Erythrocyte Membranes in Patients with Malignant Tumors of Different Location ($X \pm m$)

| Group | Arachidonic to linoleic acid content ($C_{20:4}/C_{18:2}$) | Sum of saturated fatty acids, % | Sum of unsaturated fatty acids, % | Sum of polyunsaturated fatty acids, % | Ratio of sum of saturated fatty acids to sum of unsaturated fatty acids | Ratio of sum of saturated fatty acids to sum of polyunsaturated fatty acids |
|---|---|---|---|---|---|---|
| Donors (control) | 0.81±0.14 | 64.11±2.98 | 35.89±2.98 | 14.08±2.16 | 1.92±0.28 | 4.22±0.44 |
| | 1.78±0.35 | 57.40±3.65 | 42.60±3.65 | 14.41±1.72 | 1.47±0.24 | 5.05±0.63 |
| Patients with lung cancer | 0.91±0.25 | 71.06±4.20 | 28.94±4.20 | 5.38±1.33** | 2.86±0.57 | 9.92±5.06*** |
| | 0.96±0.48 | 60.57±3.53 | 39.43±3.53 | 10.92±2.41 | 1.57±0.28 | 8.96±2.98 |
| Patients with head and neck tumors | 0.59±0.15 | 67.84±2.77 | 32.16±2.77 | 9.63±1.43 | 2.29±0.36 | 8.09±1.31*** |
| | 0.41±0.20** | 69.25±3.94 | 30.75±3.94 | 5.48±0.58* | 2.68±0.60 | 13.40±1.58* |
| Patients with stomach cancer | 1.09±0.47 | 59.81±7.74 | 40.19±7.74 | 7.67±1.09*** | 2.40±1.53 | 11.57±2.10** |
| | 0.43±0.16** | 69.43±2.68*** | 30.75±2.68*** | 7.26±1.23** | 2.43±0.32*** | 0.64±2.38*** |
| Patients with colorectal cancer | 0.24±0.09** | 61.79±3.43 | 38.29±3.43 | 9.02±1.52 | 1.79±0.32 | 8.96±2.28 |
| | 0.58±0.18*** | 59.09±2.31 | 40.91±2.31 | 9.13±1.70 | 1.49±0.13 | 8.72±2.21 |

**Note.** Numerator: parameters of fatty-acid spectrum of phosphatidylcholine fraction; denominator: phosphatidylethanolamine fatty acid spectrum. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to donors (control).

**TABLE 3.** Pyrene Probe Fluorescence Parameters in Erythrocyte Membranes of Patients with Malignant Tumors of Different Location ($X \pm m$)

| Group | Fluorescence parameters | | | | | |
|---|---|---|---|---|---|---|
| | $J_{370}/J_{470}$ ($\lambda_{ex}$=285 nm), arb. units | $J_{390}/J_{470}$ ($\lambda_{ex}$=285 nm), arb. units | $J_{370}/J_{470}$ ($\lambda_{ex}$=340 nm), arb. units | $J_{390}/J_{470}$ ($\lambda_{ex}$=340 nm), arb. units | $J_{370}/J_{390}$ ($\lambda_{ex}$=340 nm), arb. units | $R$, % |
| Donors (control) | 2.13±0.07 | 1.88±0.06 | 1.12±0.03 | 1.04±0.03 | 1.08±0.01 | 68.84±0.89 |
| Patients with lung cancer | 2.44±0.17**** | 2.15±0.15**** | 1.47±0.10* | 1.29±0.07* | 1.13±0.01** | 67.15±1.19 |
| Patients with head and neck tumors | 2.59±0.17** | 2.24±0.16*** | 1.38±0.04* | 1.20±0.04** | 1.14±0.01* | 70.09±1.01 |
| Patients with stomach cancer | 2.49±0.16*** | 2.18±0.14**** | 1.49±0.09* | 1.32±0.09* | 1.13±0.01* | 72.00±0.81****+ |
| Patients with colorectal cancer | 2.08±0.11° | 1.77±0.10° | 1.25±0.06**** | 1.08±0.06 | 1.16±0.01*++x | 69.70±0.76 |

**Note.** *$p$<0.001, **$p$<0.01, ***$p$<0.02, ****$p$<0.05 compared to donors (control); +$p$<0.01, ++$p$<0.05 compared to patients with lung cancer; °$p$<0.05 compared to patients with head and neck tumors; x$p$<0.05 compared to patients with stomach cancer.

detected in patients with tumors of different location can be apparently regarded as nonspecific signs of the involvement of peripheral component of the erythron in the intricate complex changes in the body, concomitant with the development of tumor process. On the other hand, we cannot neglect the fact that despite universal direction of changes in the structure and properties of the erythrocyte membrane lipid bilayer in cancer patients, the severity of the detected shifts in some parameters did depend on the tumor location (Tables 1, 3).

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