Erythrocyte Membranes in Patients with Malignant Tumors as Shown by Fluorescent Probing

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Erythrocyte membranes were examined by fluorescent probing in untreated patients with tumors of different localization (lung cancer, head and neck tumors, stomach and colorectal cancer). Tumor growth was associated with pronounced disorders in erythrocyte membranes: increased viscosity of the lipid bilayer, including protein-lipid contacts, and modification membrane surface layers. The degree of changes in the studied parameters depended on the tumor location.

Key Words: *erythrocyte; membrane; fluorescent probes; microviscosity; cancer patients*

Disorders in the morphology and function of circulating erythrocytes, in particular during tumor growth, are largely determined by physical and chemical properties of their membranes, specifically the state of membrane lipids and proteins. The structure of lipid bilayer of erythrocyte membranes and protein-lipid interactions can be evaluated using fluorescent probes. Erythrocytes are the most abundant blood cells responsible for the main vital function, respiration; they represent a unique model for evaluating the status of the whole organism. The level of structural and metabolic disorders in erythrocytes can reflect the intensity of pathological processes [3,6-8]. This prompted us to investigate the erythrocyte membranes in cancer patients (lung cancer, head and neck tumors, stomach and colorectal cancer) using fluorescent probes.

MATERIALS AND METHODS

Four groups of cancer patients (n=50) aged 43-67 years were examined. The differences in the studied parameters between male and female cancer patients and donors were statistically negligible. The group of patients with malignant tumors included patients with II-IV stage lung cancer (n=13), head and neck tumors (n=12), stomach cancer (n=16), colorectal cancer (n= 9). Cancer was in each case diagnosed by x-ray, endoscopic, and morphological methods. Cancer patients were examined before antitumor therapy. Control group consisted of 30 donors. Venous blood was analyzed.

Erythrocyte membranes were isolated as described previously [13,10]. Fluorophores were pyrene, 1-anilinonaphthalene-8-sulfonate (ANS), and phenylnaphthylamine (PNA, Sigma). When examining erythrocyte membranes using fluorescent probe pyrene, the ratios of fluorescence intensities I_{370}/I_{470} and I_{390}/I_{470} at λ_{ex}=285 and 340 nm and I_{370}/I_{390} at λ_{ex}=340 nm were evaluated. The inductive resonance energy transfer from tryptophane residues to pyrene in erythrocyte membranes was calculated as described previously [2,4,5]. Polarization of PNA fluorescence was evaluated for assessing the microviscosity of the probe microenvironment. The binding constant and the number of ANS binding sites were determined graphically by double reciprocal plot [2,5]. The significance of difference between the studied groups was evaluated by Student's t test and nonparametric tests.

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RESULTS

Pyrene, a non-polar fluorescent probe, diffuses in the hydrocarbon core and probably glycerol residues of the membrane [2,5]. Our results indicate that in erythrocyte membranes from cancer patients (lung, stomach, head and neck) the pyrene fluorescence parameters (I_{370}/I_{470} and I_{390}/I_{470} at λ_{ex}=285 and 340 nm) increased in comparison with healthy subjects. The polarity of lipid bilayer of erythrocyte membranes also increased in these patients, which was associated with a significant increase in the fluorescence parameter I_{370}/I_{390} at λ_{ex}=340 nm in comparison with that in healthy subjects. In erythrocyte membranes of patients with colorectal cancer, the pyrene fluorescence parameters I_{370}/I_{490} and I_{370}/I_{390} at λ_{ex}=340 nm increased in comparison with donors. The energy transfer efficiency was increased in erythrocyte membranes of patients with stomach cancer (Table 1).

Increased pyrene fluorescence parameters in erythrocyte membranes I_{370}/I_{470} and I_{390}/I_{470} at λ_{ex}=285 nm in cancer patients in comparison with that in healthy individuals indicates increased microviscosity or decreased hydrophobic volume of the protein-lipid contact zone [2]. The hypothesis on a decreased hydrophobic volume of the lipid phase adjacent to integral proteins and enhanced dissociation of the protein component from erythrocyte membranes in patients with stomach cancer was confirmed by a significant increase in the percentage of energy transfer from tryptophane to pyrene in these patients in comparison with healthy subjects. Increased energy transfer reflects the involvement of the protein component, which, together with dissociation or rearrangement of protein molecules, can be associated with their submerging into lipid bilayer [1,11]. These changes can contribute to increased polarity of pyrene microenvironment.

A pronounced increase in the pyrene fluorescence parameters I_{370}/I_{470} and I_{390}/I_{470} at λ_{ex}=340 nm in erythrocyte membranes of cancer patients with different location of the tumor in comparison with the same parameters in healthy subjects indicated a high viscosity of the lipid bilayer. The increase in parameters of pyrene fluorescence characterizing the structural and functional disorders in deep layers of erythrocyte membranes and their increased viscosity can be caused by appreciable changes in lipid composition accompanying tumor process [9,12].

A notable increase in I_{370}/I_{390} ratio at λ_{ex}=340 nm indicates increased polarity of pyrene microenvironment in the depth of the lipid bilayer and/or loosening of the phospholipid packing [4,5], which may result from accumulation of polar H_2O molecules in the erythrocyte membranes due to intensification of LPO processes and opening of polar groups of protein molecules during dissociation of oligomer assemblies.

Increased microviscosity of erythrocyte membrane probably resulting from LPO activation was detected in patients with lung cancer using PNA (Table 2). This probe is primarily located in the membrane at the depth of carbonyl groups and glycerol residues of phospholipids [2,5]. Increased polarization of PNA fluorescence in erythrocyte membranes of patients with lung cancer indicates impairment of the lipid bilayer structure.

Surface layers of erythrocyte membranes of cancer patients were examined with ANS. In patients with tumors of the head and neck the binding constant was decreased and the number of ANS binding sites on erythrocyte membrane increased in comparison with those in healthy subjects (Table 2). In the rest patients

TABLE 1. Parameters of Pyrene Fluorescence in Erythrocyte Membranes of Patients with Malignant Tumors of Different Localization ($X \pm m$)

Group	Fluorescence parameters, arb. units					Energy transfer index, %
	λ_{ex}=285 nm		λ_{ex}=340 nm			
	I_{370}/I_{470}	I_{390}/I_{470}	I_{370}/I_{470}	I_{390}/I_{470}	I_{370}/I_{390}	
Healthy subjects	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
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
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***++
colorectal cancer	2.08±0.11x	1.77±0.10x	1.25±0.06**	1.08±0.06	1.16±0.01***+o	69.70±0.76

Note. *p<0.02, **p<0.05, ***p<0.001 *vs.* donors; +p<0.05 and ++p<0.01 vs. patients with lung cancer; p<0.05: x*vs.* patients with head and neck tumors, o*vs.* patients with stomach cancer.

TABLE 2. Parameters of ANS Binding and PNA Fluorescence Polarization in Erythrocyte Membranes of Patients with Malignant Tumors of Different Localization ($X \pm m$)

Group		Binding constant	Number of binding sites	Fluorescence polarization
		arb. units		
Healthy subjects		0.052±0.012	4.79±1.28	0.070±0.002
Patients with	lung cancer	0.043±0.009	14.87±4.87	0.086±0.004***
	head and neck tumors	0.021±0.004*	17.17±5.62**	0.069±0.003++
	stomach cancer	0.051±0.008x	6.37±1.25	0.074±0.002+
	colorectal cancer	0.030±0.008	8.71±3.58	0.068±0.004++

Note. *$p<0.02$, **$p<0.05$, ***$p<0.001$ *vs.* donors; +$p<0.05$ and ++$p<0.01$ *vs.* patients with lung cancer; x$p<0.05$ *vs.* patients with head and neck tumors.

the parameters of ANS binding to erythrocyte membranes although similarly changed, only insignificantly differed from the respective parameters in healthy subjects. Changes in the binding constant and number of ANS binding sites probably result from modification of the probe adsorption centers in the cell membrane [2,5].

Comparison of the parameters of pyrene, ANS, and PNA fluorescence in erythrocyte membranes of patients with tumors of different locations showed differences in the degree of changes in the studied parameters (Tables 1 and 2).

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