ONCOLOGY

Surface Architectonics and Ultrastructure of Peripheral Blood Erythrocytes in Tumor Patients

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Scanning and transmission electron microscopy revealed similar changes of erythrocyte surface and ultrastructure in patients with lung, stomach and colorectal cancer, head and neck tumors. The number of discocytes was sharply reduced, the percentage of transitional, prehemolytic, and degenerative forms increased, while erythrocyte ultrastructure was often disordered.

Key Words: *erythrocytes; tumor patients; electron microscopy*

Tumor process is often accompanied by anemic syndrome. An important role in the pathogenesis of tumor-associated anemia is played by enhanced erythrocyte hemolysis due to their structural deficiency [1]. Prehemolytic erythrocyte abnormalities are associated with structural and metabolic alterations in their membranes [5] and can be identified by electron microscopy [3,4]. In light of this, the study of the structure of peripheral blood erythrocytes is of practical importance.

MATERIALS AND METHODS

Sixty-seven patients with lung cancer (stages III-IV), 36 patients with head and neck tumors (HNT: cancer of the tongue, oral cavity, and larynx, stages II-III), 30 patients with stomach cancer, and 24 patients with colorectal cancer (stages II-IV) were examined. The patients aged 40-65 years. In each case the diagnosis was confirmed morphologically, endoscopically, and by x-ray examination. Control group comprised 17 healthy individuals. Blood was drawn from finger tip after overnight fast.

Specimens for electron microscopy were prepared as described elsewhere [3] and examined under an REM-200 electron microscope (35 kW accelerating voltage, 0.63 A current, 35° slope). In each sample the diameters of erythrocytes and central hollow in 50 randomly selected erythrocytes were determined and the ratio of these parameters was calculated and expressed in percents. For evaluation of the distribution of different morphological forms of erythrocytes (according to classification [2,3]), 1000 cells from each patients were counted.

Erythrocytes for transmission electron microscopy were fixed in glutaraldehyde, postfixed with osmium tetroxide, and washed with cacodylate buffer. The specimens were dehydrated in ascending alcohol concentrations (from 50 to 96%) and treated with propylene oxide for 20-30 min. The pellet was embedded in Araldite. Ultrathin sections prepared on an Ultratom III were stained with uranyl acetate and lead citrate, examined, and photographed under a JEM-100 electron microscope.

The significance of differences was evaluated using Student's t test and nonparametric tests (for non-Gaussian distribution).

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RESULTS

Scanning electron microscopy revealed different morphology and surface architectonics of erythrocytes from healthy donors. Biconcave discocytes constituted 87.04±0.22% of total erythrocytes population. Erythrocytes with reversible changes in shape, i.e. transitional forms (ellipses, flat discs, discocytes with single or multiple processes and crests, and "mulberry" erythrocytes) constituted 10.65±0.18%, prehemolytic forms (dome-shaped, spherical, "loose ball" erythrocytes) constituted 2.18±0.05%, and degenerative erythrocytes constituted 0.13±0.01%. There were no significant differences between men and women (both healthy donors and tumor patients) in the surface architectonic parameters and external to internal discocyte diameter ratio.

In all patients with lung, stomach, and colorectal cancer and HNT, the number of biconcave discocytes decreased, while the number of transitional, prehemolytic, and degenerative erythrocytes increased in comparison with that in healthy donors ($p<0.001$, Fig. 1).

The highest percentage of biconcave discocytes was found in patients with lung cancer and the lowest in patients with colorectal cancer. The percentage of transitional erythrocyte forms was practically the same in all patients, while the number of prehemolytic forms was maximum in patients with stomach cancer and minimum in patients with lung cancer. The highest percentage of degenerative forms was found in patients with colorectal cancer and the lowest in patients with stomach and lung cancer (Table 1).

Cytometry of red blood cells showed that the ratio of external to internal diameter in discocytes from tumor patients considerably surpassed the corresponding value for erythrocytes from healthy donors. The largest central hollow was found in patients with lung cancer (Table 1).

Changes in erythrocyte shape were accompanied by ultrastructural alterations, which was confirmed by transmission electron microscopy.

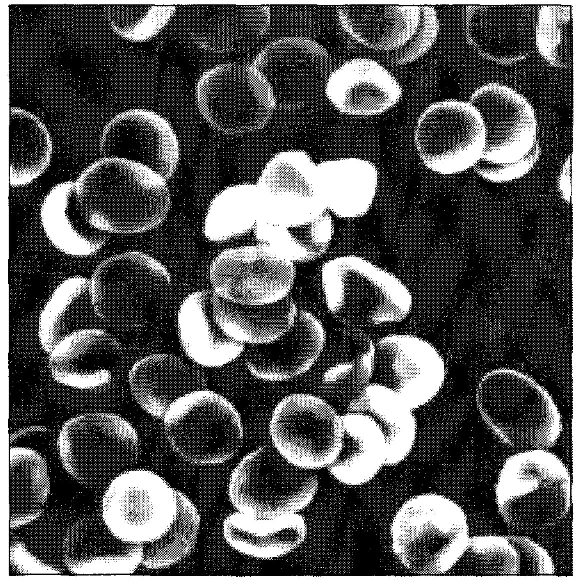

Fig. 1. Erythrocytes from a patient with colorectal cancer. Biconcave erythrocytes with single or multiple processes and crest. Scanning electron microscopy, ×1600.

It was found that erythrocytes from healthy donors had homogenous cytoplasm. Some erythrocytes contained endovesicles (lamellar and ring-shaped structures). Outer erythrocyte membrane was clearly contoured, its separation from the stroma and cavities were noted only in few cells. In some cells exovesicles and/or proteins in the primembrane space were found.

In patients with malignant tumors (lung, stomach, and colorectal cancer and HNT), the number of cells with endovesicles increased in comparison with the control (Fig. 2, *a*). Separation of the outer erythrocyte membrane was more often noted in patients with neoplasms in comparison with healthy donors. Patients with colorectal cancer and HNT did not differ in this parameter (Fig. 2, *b*), while in patients with lung and stomach cancer this increase was less pronounced. The number of red cells with endovesicles in the primembrane space increased in HNT and, to a lesser extent,

TABLE 1. Morphological Characteristics of Peripheral Blood Erythrocytes from Patients with Malignant Neoplasms of Different Localization (Scanning Electron Microscopy Data, $X\pm m$)

Patients	Morphological forms, %				External/internal diameter ratio, %
	discocytes	transitional	prehemolytic	degenerative	
Lung cancer (n=67)	79.02±0.19	15.58±0.15	4.51±0.07	0.89±0.04	57.03±0.25
HNT (n=36)	$78.19\pm0.40^{*}$	15.65±0.28	$5.14\pm0.15^{*}$	$1.02\pm0.04^{*}$	$54.60\pm0.28^{**}$
Stomach cancer (n=30)	$76.98\pm0.25^{**+}$	15.54±0.24	$6.59\pm0.09^{**+++}$	0.89±0.07	$53.90\pm0.28^{**}$
Colorectal cancer (n=24)	$76.34\pm0.30^{**++}$	15.84±0.27	$6.50\pm0.09^{**+++}$	$1.32\pm0.06^{**+++x}$	$54.50\pm0.32^{**}$

Note. $^{*}p<0.05$, $^{**}p<0.001$ compared with the corresponding parameters in patients with lung cancer; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ compared with patients with HNT; $^{x}p<0.001$ compared with patients with stomach cancer.

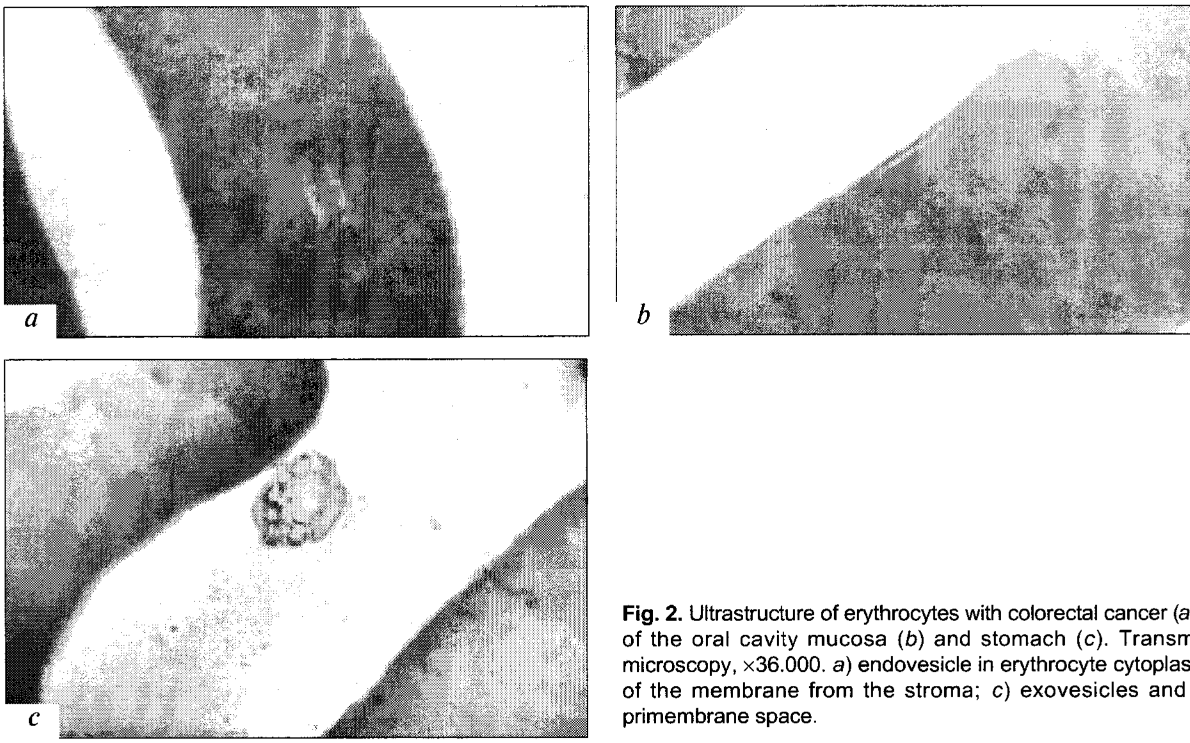

Fig. 2. Ultrastructure of erythrocytes with colorectal cancer (*a*) and neoplasms of the oral cavity mucosa (*b*) and stomach (*c*). Transmission electron microscopy, ×36.000. *a*) endovesicle in erythrocyte cytoplasm; *b*) separation of the membrane from the stroma; *c*) exovesicles and proteins in the primembrane space.

in stomach and lung cancer. The content of proteins in the intercellular space was markedly increased in patients with colorectal cancer in comparison with healthy donors and patients with lung and stomach cancer, but it remained unchanged in patients with HNT (Fig. 2, *c*).

The observed changes in surface architectonics and ultrastructure of peripheral blood erythrocytes accompanying tumor process can affect microrheological properties of the blood, promote the development of hypoxia, and aggravate patient's state.

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