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Cell Architectonics and Surface Properties of Normal Lymphocytes and Lymphoblastic Cells from Patients with Chronic Lymphoid Leukemia in the Condition of Activation and Blockade of Elements Adenylate Cyclases Signaling Pathways

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Abstract: The participation of the elements of adenylate cyclases signaling pathways in the change of properties and cell architectonics of surface in the normal lymphocytes and tumor clone lymphocytes from patients with Chronic Lymphoid Leukemia (CLL) was proved. It has been shown under influence of adrenaline the stiffness increased as for normal lymphocyte by 36.4% ($p<0.05$) and so tumor lymphocyte by 233% ($p<0.05$) as compared with control. In the relief of surface abnormal lymphocytes was observed the decrease the number of globules protrusions and increase their height but in the normal lymphocytes was increased the number of globules protrusions with reduced height. Revealed features of cell's surface properties may be used as objective criteria of functional conditions of lymphocytes during treatment of leukemia disease.

Key words: Llymphocytes, chronic lymphoid leukemia, Young's modulus, cell architectonics, element

INTRODUCTION

The development of various types of leukemia in the man occurs with participation of beta-adrenergic signaling pathways (Lamkin *et al*., 2012; Luong and Nguyen, 2012). A number of studieshave been founded the involved of beta-adrenergic receptors in the transmission of signal in the tumor cells and proved their role in the cell proliferation (Perez-Sayans *et al*., 2010), apoptosis (Sastry *et al*., 2007), metastasis (Lang *et al*., 2004), growth and angiogenesis (Shang *et al*., 2009). An important role is assigned to the search for the targeted drug action affecting to the various signaling cascade into the tumor cell (Chakraborti *et al*., 2010; Grothey and Galanis, 2009). In this connection, the one of the most promising directions in the field of biology of tumor cells is the study of properties of cell surface under influence agonists and antagonists of beta-adrenoreceptors that allow making a prognosis for future development of disease. The aim is the investigation of the participation of elements of the adenylate cyclases signaling pathways in the forming of the "picture" cell surface and elastic properties of normal ly mphocytes and lymphoid cells from patients with Chronic Lymphoid Leukemia (CLL).

MATERIALS AND METHODS

The lymphocytes of 100 donors and 50 patients with CLL were chosen as the object of study. The human blood was obtained by venipuncture. The blood was collected into vacuum tubes. The blood was centrifuged (1500 rpm) for 5 min in order to extract lymphocytes. Then, lymphocytes were rewashed of the isotonic buffer Dulbecco's solution (pH 7.4) and resuspended in Hank's medium 199. In this study was used the method of functional load *in vitro* containing the activation and blockade of receptors adenylate cyclases signaling pathways by adrenaline and propranolol.

The experimental samples were prepared as follows: 200 μL suspensions of lymphocytes were incubated into 1000 μL Hank's medium containing 10^{-9} mmol L^{-1} of adrenaline (the 1st sample) and 10^{-9} mmol L^{-1} of propranolol (the 2nd sample) for 15 min at 37°C. The sample of lymphocytes placing into the Hank's medium without adrenaline and propranolol and incubating at the same conditions as experimental samples were used as a control. After incubation the samples were centrifuged and then the supernatant were removed. The cell architecture was studied using an Ntegra Vita atomic force microscope (configuration on the basis of an Olympus

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IX-71 inverted microscope). Lymphocyte suspension was applied onto clean degreased glass plates and transferred into a humid chamber for maintain their viability. Scanning of 20 cells from each experimental and control sample was performed in a tapping mode (scan frequency 0.6-0.8 Hz) by a NSG03 cantilever with tip radius 04-10 nm and spring constant of 1.1 N/m. The obtained scans were used for construction of 3.5×3.5 μm surface profiles Nova Software (NT-MDT, Zelenograd, 2009) and morphological structures were measured on these profiles (the number and height of globules protrusions and sizes of recesses in the membrane were determined).

The elastic properties of cells were studied by method of atomic force spectroscopy. For experiments modified AFM probes like hemispheres with radius of 2.5 μm were used (Skorkina *et al*., 2011). During atomic force spectroscopy of lymphocyte surface, force curves were recorded and used for calculation of the probe-sample interaction force (Capella and Dietler, 1999). The obtained experimental data were statistically processed. significance of differences was evaluated using student t-test.

RESULTS

During incubation of normal lymphocytes with adrenaline the stiffness of cells was increased by 36.4% ($p<0.05$) as compared with control. In the relief of surface, the number of globules protrusions was increased with reducing of their height (Table 1). In the parallel experiments with propranolol the stiffness of cells decreased by 20% ($p<0.05$) and a height of globules protrusions increased but their numbers unchanged.

Observing on scans the roughness of cell surface after adrenaline load was accompanied by increase of the number of globules protrusions by 27.7% ($p<0.05$) and their height reduced by 42.4% ($p<0.05$) as compared with control. After adrenaline load was occurred the significant reduce of depressions in the membrane by 55.5 ($p<0.05$) and their diameter and depth decreased respectively by 28.7 and 32.6% ($p<0.05$) as compared with control (Table 1).

In the samples with propranolol was revealed the decrease a diameter of depressions by 50% ($p<0.05$) as compared with control. It was established the height of globules protrusions increased approximately by 4 times and their numbers reduced by 24% ($p<0.05$) as compared with adrenaline load (Table 1). The numbers of depressions in the membrane increased by 1.7 times and their diameter and height increased, respectively by

Table 1: The structural and mechanical properties of normal lymphocytes under adrenaline and propranolol loads

Parameters of the structures and properties of cell's surface	Control	Adrenaline	Propranolol
Globules protrusions			
Height (nm)	41.3±3.70	17.5±0.5*	75.8±8.8*▲
Numbers	36.0±0.90	46.0±1.1*	35.0±1.6
Depressions in the membrane			
Diameter (nm)	221.8±24.0	63.8±1.1*	110.1±1.3*▲
Depth (nm)	17.3±0.60	5.6±0.1*	13.6±1.7▲
numbers	18.0±1.10	10.0±2.3*	17.0±1.9▲
Young's modulus (μPa)	3.5±0.20	5.5±0.4*	2.8±0.3*▲

Table 2: The structural and mechanical properties of lymphoid cells from patients with CLL under adrenaline and propranolol loads

Parameters of the structures and elastic properties of cell's surface	Control	Adrenaline	Propranolol
Globules protrusions			
height (nm)	17.6±0.9	72.8±1.3*	15.7±0.9▲
numbers	125.0±0.1	26.0±0.9*	24.0±0.4*
Depressions in the membrane	149.4±12.9	9.3±1.9*	0.9±0.1*▲
Diameter (nm)			
depth (nm)	8.0±0.9	35.5±3.6*	14.9±2.9*▲
numbers	40.0±2.3	12.0±1.4*	9.0±0.2*▲
Young's modulus (μPa)	1.80±0.01	4.2±0.3*	2.6±0.4▲

*Statistically significant differences between the value in adrenaline and propranolol load compared with control by the Student's criterion at $p<0.05$; ▲Statistically significant differences between the values in propranolol load compared with adrenaline load by the Student's criterion at $p<0.05$

73 and 142% ($p<0.05$) as compared with adrenaline load. In the conditions of blockade the beta-adrenoreceptors was improved the elastic properties of membrane. The Young's modules decreased by 21% ($p<0.05$) as compared with adrenaline load samples. In the samples from patients with CLL under adrenaline load the stiffness of cells increased by 233% ($p<0.05$) as compared with control here with the numbers of the globules protrusions in the membrane reduced and their height increased. In the samples with propranolol the stiffness of lymphoblastic cells increased by 143% ($p<0.05$) and the numbers of globules reduced and their height was in the range unauthentic differences with the control (Table 2).

In the patients with CLL under adrenaline and propranolol loads the relief of cell surface was less structured than control samples (the lymphocytes from patients with CLL placed in the autologous plasma). Under adrenaline load the number of depressions in membrane reduced by 70% ($p<0.05$) and a diameter increased by 93.7% ($p<0.05$) as compared with control. The number of globules protrusions decreased by 79% ($p<0.05$) and their height increased by 314% ($p<0.05$) as compared with control (Table 2). In the samples with propranolol the number of depressions in the membrane decreased by 77.5% ($p<0.05$) their diameter reduced by 99.4% ($p<0.05$) but the depth increased by 85.7% ($p<0.05$)

as compared with control. The number of globules protrusions decreased by 80.8% ($p<0.05$) but their height almost unchanged as compared with control (Table 2).

In the conditions of blockade beta-adrenoreceptors the height of globules protrusions in the membrane of lymphoid cells from patients with CLL reduced by 78% ($p<0.05$) herewith their number was in the range unauthentic differences with the samples under adrenaline load. Under influence of propranolol a diameter, a depth and number of depressions in the membrane reduced respectively by 90.5, 58 and 25% ($p<0.05$) as compared with adrenaline load. In the conditions of blockade of beta-adrenoreceptors the elastic properties were improved. The Young's modules decreased by 61.5% ($p<0.05$) as compared with adrenaline load.

DISCUSSION

In the conditions of activation of receptors adenylate cyclases signaling pathways stiffness as normal lymphocytes so abnormal lymphoid cells significance increased but the reactions of surface health and abnormal cell differs. On the surface of tumor lymphocytes is appears the corrugations (Reduce the number of globules protrusions and increased their height) but in the surface of normal lymphocytes from donors is observed the roughness (increase the number of globules protrusions with reducing height).

One can assume that the mechanism lying in the base of the change the properties and "picture" of surface relief of tumor lymphocytes is associated with abnormal regulation into them phosphoinositide signaling transduction pathways. In the experimental work (Wymann and Schneiter, 2008) was found the change of quantitative and qualitative composition of all classes of the membrane phospholipids in the tumor cells and decrease the level of ATP on the background increase the destruction of CAMF synthesis. In the membrane of patients with CLL the activity of phosphoinositol specific phosphodiesterase is inhibited (Kazatian *et al*., 2011).

CONCLUSION

Thus in our studies have been experimentally proved the participation of elements adenylate cyclases signaling pathways in the change of properties and architecture of cell surface. In the conditions of activation of beta-adrenoreceptors as normal so abnormal lymphocytes the stiffness of cells increased. On the surface of tumor lymphocytes the number of globules protrusions reduce and their height decreased. Revealed features may be importance prognosis means in the future development of tactics increase but in the surface of normal lymphocytes the number of globules protrusions increase and their height therapy of the patients with chronic lymphoid leukemia.

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