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APPLICATION OF TECHNOLOGIES OF ATOMIC FORCE MICROSCOPE INVESTIGATION FOR EVALUATION OF STRUCTURE AND PROPERTIES OF BLOOD CELLS' SURFACES

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Abstract
With application of techniques of atomic force microscope investigation, we studied properties and topography of blood cells' surfaces in patients with acute lymphoblastic and myeloblastic leucosis. It was stated that development of acute forms of lymphoblastic and myeloblastic types of proliferation is accompanied with reduction of stiffness of cell surface and increase of surface potential. It was proved that in remission state of acute lymphoblastic leucosis, cells preserve abnormal properties of surface, which are typical for acute stages of disease. However, topography of surface of blood cells significantly differs both among various types of leucosis and at various stages of disease course (aggravation or remission). Thus, usage of method of force microscope investigation allows visualizing early developments of malignant transformation of blood cells, and it may be recommended as one of objective criteria of effectiveness of therapy at cancer disease.
Key words: acute lymphoblastic leucosis, acute myeloblastic leucosis, Young's modulus, surface potential, topography of surface.

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ИСПОЛЬЗОВАНИЕ ТЕХНОЛОГИЙ АТОМНО-СИЛОВОЙ МИКРОСКОПИИ ДЛЯ ОЦЕНКИ СТРУКТУРЫ И СВОЙСТВ ПОВЕРХНОСТИ КЛЕТОК КРОВИ

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Аннотация
С использованием технологий атомно-силовой микроскопии изучены свойства и топография поверхности клеток крови больных острым лимфобластным и миелобластным типом лейкоза. Установлено, что развитие острых форм миело- и лимфобалстного типа пролиферации сопровождается снижением жесткости клеточной поверхности и увеличением поверхностного потенциала. Доказано, что на стадии ремиссии острого лимфобластного лейкоза клетки сохраняют аномальные свойства поверхности, характерные для острых стадий течения болезни. Однако топография поверхности клеток крови существенно отличается как между различными типами лейкоза, так и на стадиях обострения и ремиссии. Следовательно, использование метода атомно-силовой микроскопии позволяет визуализировать ранние развития опухолевой трансформации клеток крови и может быть рекомендовано в качестве одного из объективных критериев эффективности проводимой терапии при онкологических заболеваниях.
Ключевые слова: острый лимфобластный лейкоз, острый миелобластный лейкоз, модуль Юнга, поверхностный потенциал, топография поверхности.

Promising direction in the sphere of biomedicine is application of technology of atomic force microscope investigation for finding criteria that allow reasonably evaluate effectiveness of conducted antitumor therapy at the cellular level. Atomic force microscopy is a universal technique for measuring powers in nanoscopic levels. By now, characteristics of mechanical contacts between cells at the level of one molecule have been described [4, 5], modifications of force spectroscopy (FS) mode have been developed, which allow studying receptors of cell surface [6].

One of the promising directions with application of AFM is study of elastic features of cell surface, which gives the possibility to quantitatively evaluate local elasticity modulus, as well as adhesive strength, by means of visualizing of shearing elastic characteristics of biological tissues [8]. Sensitivity of FS method depends on cantilever's characteristics. For the work with inert objects in FS mode, it is recommended to use probes with "soft bar", stiffness constant of which varies from 10^{-2}-10^{-1} N/m and rounded radius of 10 nm [7]. The subject of the work is to study peculiarities of structure and properties of blood cell surfaces at development of tumor processes in blood system.

Materials and methods of the research

In this work, peripheral blood of patients with various types of leukemia was used. Diagnosis was made for the first time and any treatment had not been introduced. The disease was diagnosed in clinical and diagnostic laboratory of St. Ioasaf Regional Hospital in Belgorod. For the experiment, whole blood samples were selected, taken from patients with acute lymphoblastic leucosis in the exacerbation phase (n=10) and remission of the disease (n=10), acute myeloblastic leucosis (n=8). For control, blood of healthy people was used (n=28). For comparison, we used two rows of data: normal parameters of lymphocytes, and lymphocytes in condition of development of lymphoproliferative processes in blood system. Blood was obtained by means of vein puncture with participation of qualified medical staff. Samples were collected into vacuum tubes Vacuette K3E. Tumorous clones of cells from lymphoid and myeloid lines were extracted from whole blood by means of centrifugation at 1500 rpm within 5 min with further three-stage washing with RPMI-1640 medium and re-suspending in the same medium.

Before conduction of experiments, we performed tests for assessment of cell viability, by means of coloring of 1 mcl of suspended mixture with 0.4% solution of trypan blue substance in phosphate-buffer saline (pH 7.2-7.3) and further calculation of died forms with the help of light microscope. In this experiment, we used samples with cell vitality of not less than 98%.

Study of elastic properties of blastic forms of blood was performed at atomic force microscope INTEGRA Vita (configuration on the base of inverted optical microscope OlympusIX-71, produced by NTMDT, Zelenograd, 2009). Medications were

prepared by means of application of native cell suspended mixture onto glass degreased base coats. Scanning of cells was performed in the mode of force spectroscopy with application of cantilever NSG03. 20 cells were scanned from each sample. With the help of Nova software, we applied loading in 25 areas of cell surface. Obtained force curves of supply and removal were processed on the base of Hertz model and Sneddon modification with the help of software 'Ef3' (NT-MDT, Zelenograd, 2009).

Electrical features of tumor cells' membranes were measured in the mode of Kelvin's probe. Suspension of cells for measuring surface potential (SP) were prepared according to the method, described above [3]. Measuring of SP was performed with the help of cantilever with electrically conducting titanium coating of NSG03/TiN series (Nanoworld, USA). Twenty cells were scanned from each sample, and obtained scans were processed with the help of Nova program (NT-MDT, Russia).

Relief of the surface was studied in the tapping mode of scanning. Suspended solutions of tumor cells were prepared by means of applying a drop of suspended mixture in degreased microscope slide [2]. With the aim of preserving cell vitality, they were placed into moisture cabinet before scanning [1]. Scanning was performed with scan rate of 0.6-0.8 Hz, with application of cantilever of NSG03 with stiffness of 1.1 N/m and rounded radius of 10 nm. Twenty cells were scanned from each sample. Obtained scans were used to build profile curves of surface area with the size of 3.5 x 3.5 μm, at which height was measured and number of globular projections was calculated, as well as the number of cavities that were formed in membrane after the load application.

The results of experimental studies were processed by variation statistics methods. Experimental data are presented in the forms of arithmetic means values with their average standard errors. Statistical analysis of results of the studies was performed with application of Student's criterion for 5% level of significance.

Results of the study and their discussion

As a result of experiments conducted, we established the change of charge and elastic features of cell surface at development in blood system of lymphoproliferative and myeloproliferative processed. For myeloblasts of patients with AML reduction of Young's modulus by 61.5% ($p<0.05$) is typical, in comparison with similar parameters of mature neutrophils. Charge of cell surface of myeloblast increased by 46.4% ($p<0.05$) in comparison with neutrophil's charge (Table 1).

Table 1

Values of Young's modulus and surface potential of blood cells

Samples	Young's modulus, mPa	Potential of surface, mV
Lymphocytes in norm	3.5±0.2	-37.3±0.6
Neutrophils in norm	4.8±0.5	-35.4±0.3
Myeloblasts of AML	1.85±0.3 [b]	- 24.18±0.3[b]
Lymphoblasts of ALL	1.77±0.2 [a]	- 28.36±0.1[a]
Lymphocytes of ALL in remission	2.58±0.1 [a]	- 29.43±0.3[a]

Notes: [a] *Statistically significant differences between values of cells of lymphoid line in norm and at development of ALL and AML according to Student's criterion at p<0.05.*
[b] *Statistically significant differences between values of cells of myeloid line in norm and at development of AML according to Student's criterion at p<0.05.*

At development of lymphoproliferative processes in blood system, Young's modulus of lymphoblasts of patients with ALL was reduced by 49.4% ($p<0.05$), the charge of cell surface was increased by 31.5% ($p<0.05$) in comparison with lymphocytes in norm. However, in patients' blood after the course of standard chemotherapy at remission stage, the index of lymphocytes' stiffness stayed reduced by 26.3% ($p<0.05$), and the charge of surface was increased by 26.7% ($p<0.05$) in comparison with normal lymphocytes.

Studying morphology of cellular forms at development of various types of leukemia, we stated that surface of both blasts and cytes was represented by globular projections and large invaginations of plasma membrane. In the group of patients with AML at the surface of myeloblasts the number of globular formations and their height increased by 136.7% ($p<0.05$) and 287.8% ($p<0.05$), respectively, in comparison with corresponding formations at the surface of neutrophils; at the same time, the number of cavities reduced by 89% ($p<0.05$), and their diameter was reduced almost twice (Table 2).

Table 2

Peculiarities of surface relief of blood cells

Sample	Globular projections		Cavities in membrane		
	Height, nm	Number	Diameter, nm	Depth, nm	Number
Lymphocytes in norm	41.3±3.7	36.0±0.9	221.8±24.0	17.3±0.6	18±1.1
Neutrophils in norm	36.4±2.7	37.0±0.2	217.5±19.6	15.8±0.3	23.0±0.8
Myeloblasts of AML	141.18±5.67[b]	87.6±0.5 [b]	103.43±4.21[b]	16.25±0.62	2.0±0.05 [b]
Lymphoblasts of ALL	125.40±5.1	7.0±0.5[a]	118.19±8.3	23.95±1.1	35.0±2.4[a]
Lymphocytes of ALL in remission	12.0±0.7 [a]	5.0±0.2 [a]	105.0±0.3 [a]	27.5±0.7 [a]	3.0± 0.3 [a]

Notes: [a] *Statistically significant differences between values of cells of lymphoid line in norm and at development of ALL and AML according to Student's criterion at p<0.05.*
[b] *Statistically significant differences between values of cells of myeloid line in norm and at development of AML according to Student's criterion at p<0.05.*

At the surface of lymphoblasts of patients with ALL the number of globular structures was reduced by five times, and their height was increased by 203.6% (p<0.05) in comparison with similar structures at the surface of normal lymphocytes. The number of cavities at the surface of lymphoblasts increased almost by 2 times, however, the sizes of these structures was not statistically different from similar parameters of lymphocytes' surface (Table 2).

In remission of the disease, when in the blood of patients with ALL lymphocytes are circulating, the number of globular structures and their height stayed reduced by 86% и 71% (p<0.05), respectively, in comparison with similar parameters in norm. The number of cavities on the surface of membrane was reduced by 6 times, their diameter reduced by two times, and their depth increased by 59% (p<0.05) in comparison with the norm.

Conclusion

After the analysis of the obtained data, it was stated that myoblasts and lymphoblasts at development of acute forms of leukemia are characterized by reduced stiffness of cellular surface. Such soft surface allows them quickly disseminating in blood stream and migrating into tissues. Abnormal features are preserved by lymphocytes in the group of patients with ALL at remission stage. In spite of lack of blast forms in blood, lymphocytes of these patients preserve "soft" surface and increased charge of cellular membrane. Topography of surface of blood cells vary greatly between various types of leucosis and stages of its development. In particular, development of acute myeloblastic leukemia is characterized with increased number and height of globules at cell surface, while acute lymphoblastic leukemia is accompanied with reduction of globular structures and increase of their height.

At the same time, at obtainment of long-lasting remission in organisms of patients with acute lymphoblastic leucosis, relief of lymphocytes' surface is smoothed, and the number of globular formations and their height are reduced. Thus, technologies of atomic force microscopy allow visualizing objective indicators of development of pathological processes in organism (tumor transformation of cells), and they may be used as criteria of effectiveness of conducted therapy at oncology diseases.

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