DEPENDENCE OF ADHESIVE PROPERTIES ON Ca^{2+}-ATP-ASE ACTIVITY IN TUMOR CELLS

A. V. Mtskhvetadze, M. A. Aivazishvili, I. G. Shurgaya, and T. É. Akhvlediani

UDC 576.385.5:576.526].08

KEY WORDS: tumor cells; ionic homeostasis; cohesion

The properties of intercellular junctions are directly determined by bivalent ions and specific macromolecules forming the intercellular cement. Changes in the extracellular Ca^{2+} concentration lead to disturbance of ionic homeostasis of the cell and, at the same time, affect the cohesion of the cells. We also know that cohesion is one of the most informative parameters of the state of a tissue with respect to proliferation and the formation of its resistance to tumor development. Reduction of the strength and stability of cohesion of the cells is regularly observed in connection with the onset and progression of tumors [2, 5-8].

The main aim of this investigation was to establish the connection between changes in ionic homeostasis and cohesion, i.e., to discover disturbances of that connection which result in changes in the adhesive properties of cell surfaces.

We showed previously [9] that an increase in the Ca^{2+} ion concentration in the incubation medium of Ehrlich's carcinoma cells leads to increased Ca^{2+}-ATPase activity and an increase in Ca^{2+} release from the cells. In the present investigation we used the modifying influence of ionizing radiation to discover whether a similarity exists between changes in Ca^{2+}-ATPase activity and cohesion of tumor cells.

EXPERIMENTAL METHOD

Tumor cells of a Lewis lung carcinoma and cells of Ehrlich's carcinoma (ascites variant) were used as the test objects. The experiments on Lewis lung carcinoma were performed on the 14th-15th day, and on Ehrlich's carcinoma on the 7th-8th day after transplantation. Before and after transplantation the noninbred albino mice were kept on the standard animal house diet of the Oncologic Scientific Center, Ministry of Health of the Georgian SSR. Mice with transplanted tumors were subjected to total x-ray irradiation on the RUM-17 apparatus 15 min and 20 h before the experiment began. The conditions of irradiation were: 200 kV, 10 mA, filter 1 mm Al, dose rate 50 cGy/min.

Cohesion was determined on Lewis carcinoma cells by the method in [10]: the force at which the cell was detached from the tissue fragment was recorded. The lung fragment was fixed with gelatin to a slide and its edge, with the pulmonary capsule cut off, was immersed in Hanks' solution. Under the microscope, with the aid of a micromanipulator the peripheral cells were pierced by a glass needle and detached from the fixed tissue fragment. Bending of the needle at the time of detachment of the cell was recorded by means of an ocular micrometer. The degree of bending of the needle (the end of which was drawn out on a Fonbrune microforge until a diameter of not more than 1 μ was reached) was calibrated beforehand by means of microweights. The angle of bending of the needle at the time of detachment of the cell was determined and the cohesion of the cells determined by means of calibration curves, in dynes/cell.

Oncologic Scientific Center, Ministry of Health of the Georgian SSR, Tbilisi. (Presented by Academician of the Academy of Medical Sciences of the USSR M. M. Khananashvili.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 7, pp. 85-87, July, 1991. Original article submitted April 6, 1990.

0007-4888/91/0007-1017$12.50

TABLE 1. Changes in Cohesion (in dynes/cell) and Ca^{2+}-ATPase Activity (in μmoles P/mg protein/h) 15 min after Irradiation (M ± m)

Dose, cGy	Cohesion, $\times 10^3$	Ca^{2+} ATPase activ. in Lewis carc. cells	Ca^{2+}-ATPase activity in Ehrlich's carcinoma cells
Control	56±2 (0,85)	3,1±0,1	3,0±0,3
10	147±7 (0,82)	3,5±0,1	3,3±0,1
150	67±2 (0,91)	2,1±0,2	2,3±0,1
300	55±2 (0,79)	1,1±0,1	1,8±0,1
600	25±1	0,7±0,1	1,3±0,1

Legend. Here and in Table 2 value of coefficient of correlation between cohesion and Ca^{2+}-ATPase activity in Lewis carcinoma indicated between parentheses.

TABLE 2. Changes in Cohesion (in dynes/cell) and Ca^{2+}-ATPase Activity (in μmoles P/mg protein/h) 20 h after Irradiation (M ± m)

Dose, cGy	Cohesion, $\times 10^3$	Ca^{2+} ATPase activ. in Lewis carc. cells	Ca^{2+} ATPase activ. in Ehrlich's carc. cells
Control	56±2 (0,85)	3,1±0,1	3,0±0,3
10	49±2 (0,90)	2,5±0,1	2,3±0,1
150	54±1 (0,81)	2,1±0,1	1,3±0,3
300	49±2 (0,86)	0,9±0,1	0,8±0,1
600	33±2 (0,81)	0,2±0,1	0,1±0,1

Cohesion of the Lewis lung carcinoma cells and their Ca^{2+}-ATPase activity and also its activity in Ehrlich's ascites carcinoma cells were determined after incubation for 15 min in Ringer's solution of the following composition (in mM): 140 NaCl, 8 KCl, 3 $MgCl_2$, 9.5 $CaCl_2$, and 40 Tris-HCl (pH 7.0).

Ca^{2+}-ATPase activity was determined as the difference between total and Mg^{2+}-dependent activity, and total ATPase activity was determined in medium containing (in mM): 3 ATP, 3 $MgCl_2$, 100 KCl, 0.5 EDTA, 9.5 $CaCl_2$, 30 Tris-HCl (pH 7.0). The reaction was started by adding 10-50 μg protein to the sample in a volume of 1 ml and it was stopped by adding 14% TCA. Components were added to the control samples in the opposite order, to exclude nonenzymic hydrolysis of ATP. The inorganic phosphate concentration was determined by the method of Fiske and Subbarow [11].

Statistical analysis of the data included determining the mean square deviation and calculating the coefficient of correlation between cohesion of the cells and Ca^{2+}-ATPase activity in parallel series of experiments on Lewis lung carcinoma cells.

EXPERIMENTAL RESULTS

It was found that 15 min after irradiation in a dose of 10 cGy there was a very small but statistically significant increase both in cohesion and in Ca^{2+}-ATPase activity (in cells of Lewis and Ehrlich carcinomas) (Table 1). This fact can be explained, in our view, from the standpoint of the author [1] who showed that preliminary irradiation in a dose of 10 cGy has a modifying action, manifested as an increase in the strength of inhibition, a marked increase in the intensity of

absorption of the tumors, and a decrease in the number of animals dying toward the end of observation. A further increase in the dose (150, 300, and 600 cGy) led to weakening of correlation compared with initial data for both parameters.

The data given in Table 2 do not differ in principle from those described above, except that irradiation in a dose of 10 cGy also leads to reduction of both parameters, as also with higher doses. Incidentally, the coefficient of variation of the data for measurement of cohesion varied between 2 and 6%.

Thus with an increase in the dose of radiation a general tendency was found for the cohesion of the cells and Ca^{2+}-ATPase activity to decrease.

It follows from [3, 4] that the adhesive properties of the cells are closely linked with the calcium concentration in the medium, i.e., as a rule hypocalcemia is accompanied by a decrease in cohesion and by a disturbance of the structure of the cell junctions. An increase in the intracellular Ca^{2+} concentration, which usually occurs as a result of malignant change in the cell, in our opinion [9] leads to stimulation of Ca^{2+}-ATPase activity and active transport of Ca^{2+}, which is accompanied by increased release of this ion into the intercellular space and an increase in cohesion. After exposure to ionizing radiation, Ca^{2+}-ATPase activity decreases and release is reduced, which is directly reflected also in cohesion of the tumor cells.

During malignant transformation of the cells there are evidently several mechanisms responsible for the change in their adhesive properties. The integral effect is expressed as a decrease of cohesion of the cells. but this does not contradict the possibility that a mechanism aimed in the opposite direction may exist.

LITERATURE CITED

1. G. S. Kalendo, Early Reactions of Cells to Ionizing Radiations and Their Role in Protection and Sensitization [in Russian], Moscow (1982), pp. 89-90.
2. A. G. Malenkov and E. A. Modyanova, Biofizika, **32**, No. 6, 1033 (1987).
3. A. G. Melikyants, Biofizika, No. 5, 834 (1976).
4. A. G. Melikyants, A. G. Malenkov, and A. M. Melikyan, Biofizika, No. 3, 468 (1977).
5. E. A. Modyanova, O. A. Bocharova, and V. F. Ushakov, Byull. Éksp. Biol. Med., No. 4, 459 (1980).
6. E. A. Modyanova, N. N. Kasatkina, O. A. Bocharova, and N. Yu. Tomilina, Éksp. Onkol., No. 5, 62 (1982).
7. E. A. Modyanova, O. A. Bocharova, and A. G. Malenkov, Éksp. Onkol., No. 3, 39 (1983).
8. E. A. Modyanova, Éksp. Onkol., No. 2, 30 (1988).
9. A. V. Mtskhvetadze and I. G. Shurgaya, Soobshch. Akad. Nauk Gruz. SSR, **125**, No. 1, 117 (1987).
10. D. A. Coman, Cancer Res., **4**, 625 (1944).
11. C. H. Fiske and T. Subbarow, J. Biol. Chem., **66**, 375 (1925).