QUANTITATIVE PRINCIPLES OF REDISTRIBUTION OF CONCANAVALIN A RECEPTORS ON THE SURFACE OF NORMAL AND TRANSFORMED CELLS

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UDC 616-006-018.11-076.5

KEY WORDS: fibroblasts; lamelloplasm; concanavalin A.

During contact interaction between normal cells and substrates, spreading takes place and the cell sends out processes and forms a lamellar cytoplasm or lamelloplasm [1-3]. Tumor cells spread much less than normal cells and form a defective lamelloplasm, which is usually smaller than that of normal cells [4]. The lamelloplasm consists of the peripheral, very thin part of the cytoplasm, which contains no granules visible in the light microscope: It is in this area that the sites of attachment of the cell to the substrate are located. The surface of the lamelloplasm differs in its properties from the rest of the cell surface [2, 3]. One of these properties is the ability to move its receptors, attached by a ligand, in a particular direction. After exposure to polyvalent ligands the corresponding receptors conglutinate into groups (so-called patches), which then move away selectively from certain parts of the surface (capping or clearing) [6]*. On the basis of previous visual immunologic investigations it was suggested that it is this zone of lamellar cytoplasm which is cleared in spread-out fibroblasts [1, 4, 5].

The object of this investigation was a quantitative comparison of the ratio between the area cleared of groups of receptors conglutinated with concanavalin A (CA) and the area of the lamelloplasm in normal and transformed cells.

EXPERIMENTAL METHOD

Four types of cells were used: normal mouse fibroblasts (NMF) at the second passage, transformed mouse fibroblasts of line L, a line of normal rat kidney cells (NRK), and a line of the same cells transformed by mouse sarcoma virus (Kirsten strain) – Ki-MSV. The cells were grown on coverslips by the standard method for 24 h. CA receptors on the surface of the cells were revealed by the indirect immunofluorescence method. Prefixed cells were incubated successively for 10 min in the following solutions: 1) fixative, 2) CA (50 μg/ml), 3) rabbit anti-CA-γ-globulin, 4) fluorescein isothiocyanate conjugated with donkey antirabbit γ-globulin (FITC). Living cells were incubated successively for 10 min in the following solutions (short-term incubation with CA): 1) CA, 2) anti-CA-γ-globulin, 3) FITC, 4) fixative. During long-term incubation with CA, living cells were incubated successively in the following solutions: 1) CA (10 min), 2) culture medium (60 min), 3) anti-CA-γ-globulin (10 min), 4) FITC, 5) fixative.

The cells were fixed with 4% formaldehyde solution. The luminescence photomicroscope used was from Opton. Each cell was photographed in phase contrast and in UV light. The total area of the cell which it occupied on the glass and the area of the lamelloplasm – the peripheral translucent part of the cytoplasm which contained no inclusions – was determined by means of a planimeter on photographs taken in phase contrast. The zone of clearing, i.e., the area of surface from which receptors were removed, was determined on photographs taken in UV light. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the area of the cell on the glass and also the relative area of the lamelloplasm were smaller for transformed L and Ki-MSV cells than for the corresponding normal NMF and NRK

*There is no reference 6 in the Russian original – Consultants Bureau.

Interfaculty Problem Laboratory for Molecular Biology and Bioorganic Chemistry, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 6, pp. 741-743, June, 1980. Original article submitted October 16, 1979.

0007-4888/80/8906-0843$07.50

TABLE 1. Results of Quantitative Investigation of Ratio between Areas of Lamelloplasm and of Zone Cleared from CA-Conglutinated Receptors in Normal and Transformed Cells (M ± m)

Type of cells	Prefixed cells (I)		Short-term incubation of cells with CA (II)			Long-term incubation with CA (III)		
			phase contrast		UV light	phase contrast		UV light
	S of cells, μ^2	% of lamelloplasm	S of cells, μ^2	% of lamelloplasm	% of surface cleared	S of cells, μ^2	% of lamelloplasm	% of surface cleared
НМФ	3743±516	55±3,5	2713±187	58±4	4±0,8	2298±214	55,5±1,7	45,1±2,5
L	1770±188,7	33,5±3	1661±256,6	33,9±3,3	19,4±3,2	1158±136	30,4±3,5	29,1±2,3
NRK	4350±790,7	49,9±3,8	3906±721	39,2±4,5	12±3	2851±728,9	50,3±3,2	44,4±6
Ki—MSV	1572,8±125	23,2±3	1250,7±121,5	24,5±4,8	10,45±3,1	1336,6±111,9	20,6±1,9	13,0±0,97

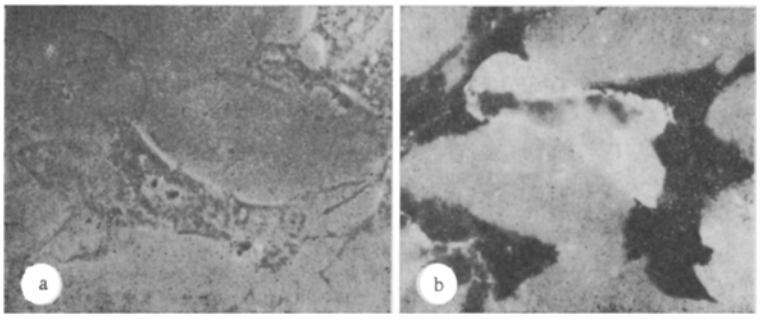

Fig. 1. Normal mouse fibroblast: a) phase contrast, 640×; b) clearing of surface of lamelloplasm from conglutinated receptors after long-term incubation with CA, 640×. Here and in Figs. 2 and 3: staining by the indirect fluorescence method.

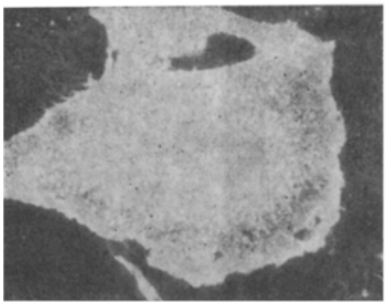

Fig. 2

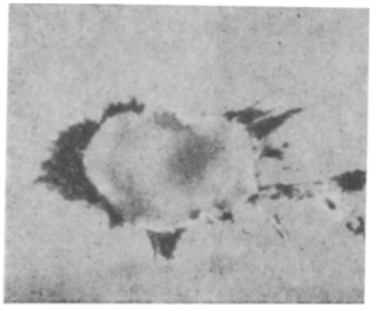

Fig. 3

Fig. 2. NRK cells. Distribution of receptors on surface of prefixed cell, 500×.

Fig. 3. L cells. Surface of lamelloplasm cleared of conglutinated receptors after long-term incubation with CA, 675×.

cells ($P < 0.05$; Table 1, column I). These results confirm observations showing that transformed cells spread to a lesser degree on the substrate than normal cells. Incubation of the living cultures with CA and the subsequent processing led to a decrease in area of the cells, but the relative dimensions of the lamelloplasm were unchanged ($P \gg 0.05$), and only in the L cells was there a further reduction in the relative area of the lamelloplasm ($P < 0.05$; Table 1, columns II and III).

On the surface of the prefixed cells CA receptors were distributed diffusely and uniformly. On incubation of the living cells with CA, zones of the surface of the lamellar cytoplasm were cleared of conglutinated receptors (Figs. 1-3).

It will also be clear from Table 1 (column III) that the dimensions of the zone cleared of patches of CA receptors were about equal to the dimensions of the lamelloplasm in normal cells ($P \gg 0.5$) and a little smaller in the transformed cells ($P < 0.02$).

Differences between the area of the lamelloplasm and area of clearing are evidently explained as follows. The transformed cells often formed long processes. The receptors were cleared only from the surface of small lamellae located at the end of these processes. The surface of the remainder of the processes, which had stable edges, was not cleared although their cytoplasm contained no granules and, for that reason, during quantitative measurements they were regarded as lamelloplasm. The reduction in the area of the lamelloplasm of the transformed cells had the result that the zone of clearance reached its maximal size during short-term incubation of these cells with CA. In normal cells, however, the zone of clearance reached its maximum during long-term incubation with CA. A further increase in the incubation time to 2 h caused no change in the dimensions of the zone of clearance.

It was thus shown that the zone of clearance from conglutinated receptors corresponded to the area of lamellar cytoplasm in normal NMF and NRK cells, but was a little smaller than the area of the lamelloplasm in transformed L and Ki-MSV cells.

Reduction of the clearance zone was characteristic of the transformed cells studied. Further investigations are necessary to determine to what extent this property is common to other types of transformed cells.

The mechanism lying at the basis of differences between the lamelloplasm and other parts of the cytoplasm remains unexplained. It can be tentatively suggested that an important role in the movement of receptors is played by microfilaments located in the cortical layer of the lamelloplasm, and that the character of interaction between the microfilaments and receptors differs in different parts of the cell cytoplasm [2, 3].

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ELECTRON-MICROSCOPIC AND HISTOCHEMICAL CHARACTERISTICS OF HEPATOMAS ARISING AFTER PROLONGED ADMINISTRATION OF CARBON TETRACHLORIDE

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UDC 616.36-006-02:547.412.133]-008.9

KEY WORDS: CCl_4; hepatomas; ultrastructure.

During prolonged injection of CCl_4 into animals, against the background of degenerative and regenerative changes in the liver, in 100% of cases hepatomas appear and gradually increase in size, turning into large tumor nodules. The ultrastructural data on the structure of these hepatomas are not numerous, and it was accordingly decided to investigate this problem. Experiments were carried out on 40 noninbred mice receiving injections of 0.2 ml of a 40% solution of CCl_4 in peach oil twice a week for 13 months. The animals were killed 7, 8, 10, 11, 12, and 13 months after the beginning of CCl_4 administration. The following methods of light microscopy were used: staining with hematoxylin and eosin, with picrofuchsine, impregnation with silver by Gomori's method, staining for fat with Sudan III, Brachet's reaction with ribonuclease control, PAS reaction with amylase.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 6, pp. 744-747, June, 1980. Original article submitted November 13, 1979.