IMMUNOMORPHOLOGIC INVESTIGATION OF CHANGES IN FOCAL CONTACTS BETWEEN CELLS TRANSFORMED BY KIRSTEN SARCOMA VIRUS IN CULTURE

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Adhesion of normal cells to substrates takes place through specialized structures known as focal contacts (FC) [1, 4, 6]. FC are detected by electron microscopy [5] or immunomorphologically [9]. The method most widely used is that of interference-reflecting microscopy [10].

Complete disappearance of FC has been found in some transformed cells, characterized by disturbance of adhesion [4]. Other investigators [7] have noted that FC are present, although in much smaller numbers, in transformed cells.

The new immunomorphologic method of antibody exclusion [11] possesses higher and finer resolution than the method of interference-reflecting microscopy usually used. By means of this method, in the investigation described below, the character of changes accompanying transformation of NRK cells by Kirsten sarcoma virus, carrying the rat oncogene, was studied. By studying cells transformed both by the wild types of virus and by its thermosensitive mutant, changes in FC of cells of the same line were able to be compared at different levels of expression of the product of the virus oncogene.

EXPERIMENTAL METHOD

The following cell lines were used: NRK, a line of kidney fibroblasts obtained from a parimary culture of rat kidney of the Osborn-Mendel line [8]; KNRK, cells of the NRK line transformed by Kirsten sarcoma virus [2]; tsKNRK, cells of the NRK line transformed by a thermosensitive mutant of Kirsten sarcoma virus. This line was obtained from Aaronson's laboratory, National Cancer Institute, Bethesda, USA, in 1976 [3]. The cells were cultured in Ham's medium F-12, containing 10% embryonic calf serum, at 32°C (the permissive temperature) and 40°C (a nonpermissive temperature). Cells for the experiments were grown on coverslips for 2 days with an initial seeding density of $4.5 \cdot 10^3$ cells/cm^2.

To detect FC two methods were used: an immunomorphologic method suggested by Neifakh [11] and interference-reflecting microscopy [10]. During culture of the cells in medium with serum, some proteins are adsorbed on the substrate. The authors obtained monoclonal antibodies to one such protein, protein 80K. With the aid of these antibodies the whole substrate except regions of FC, where the antibodies do not penetrate, are stained. The FC appear as dark spots against a light background. When the cells do not spread out well, not the all the FC can be seen because of luminescence of the central part of the cytoplasm, which is thicker. To overcome this difficulty the cells were treated beforehand with saponin and removed mechanically. Only areas of the membrane corresponding to FC, which can also be detected with the aid of monoclonal antibodies against protein 80K [12], remain on the surface of the substrate.

A Zeiss Photomicroscope III (West Germany) was used.

EXPERIMENTAL RESULTS

With the aid of antibodies against protein 80K punctate (0.2 μ or less) and elongated (0.1-0.15 × 1-1.5 μ) FC was discovered in NRK cells. The contacts were seen over the whole of the lower surface of the cells, but most were concentrated in a peripheral zone 2-4 μ wide. FC were distributed singly or were joined into short (1-2 μ), twisted chains, perpendicular to the border, forming either an interrupted network, or two or three rows parallel to the

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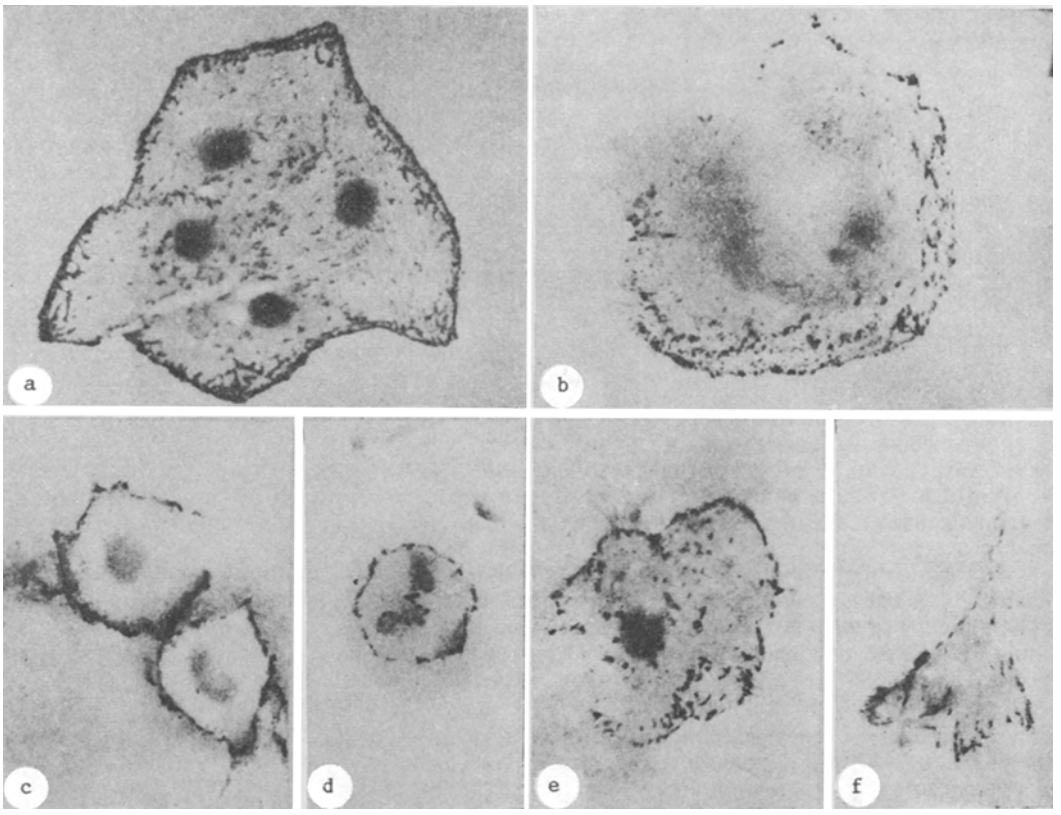

Fig. 1. Immunofluorescence with antibodies against protein 80K (black regions correspond to FC). a) Group of NRK cells: small FC located mainly in peripheral zone as a network along free borders of cells; contacts along contacting borders and in center of cells are arranged singly; b) NRK cell treated with saponin: arrangement of FC similar to that in untreated cells; c) KNRK cell, punctate FC along border; d) KNRK cells treated with saponin, punctate FC along border and in center; e) tsKNRK cell at nonpermissive temperature (40°C): punctate and elongated FC; f) tsKNRK cells at permissive temperature (32°C): reduced number of punctate and elongated FC. 1700×.

cell border. Along the border itself the contacts almost merged into a thin (0.2 μ) line. The location of the contacts described above is characteristic of the free border of the cells. Along the contacting border no concentration of contacts was observed, or it was very slight.

Contacts beneath the central part of the cells were arranged relatively uniformly, including under the nucleus (Fig. 1a, b).

The FC were preserved in KNRK cells. Only punctate contacts were found (0.2 μ or less), and their number was sharply reduced. Contacts were seen only at the periphery, singly as a rule, and less frequently they formed a broken line along the stretched lateral border of the cell (Fig. 1c).

Modification of the technique using saponin revealed a few punctate FC in the center of the cells (Fig. 1d). The general scheme of arrangement, and the size and shape of the contacts were the same in cells untreated and treated with saponin, but in the latter case the FC were evidently smaller.

Interference-reflecting microscopy gave a similar but less detailed picture of the distribution of FC. During interference microscopy it was difficult to draw a sharp line between the black areas of FC and the surrounding gray areas of the tight junctions. The reticular structure of the contacts could not be detected at the periphery of NRK cells. Small black dots of FC of KNRK cells could be observed often, if shown to be present beforehand by the immunomorphologic investigation. FC of tsKNRK cells at the nonpermissive temperature were

similar to those of NRK cells, but their distribution was more uniform and they formed a narrow (1-2 μ), less dense peripheral band (Fig. 1e).

With a change to the permissive temperature the number of contacts in the tsKNRK cells decreased sharply, and they remained only along the cell border; both punctate (0.2 μ and less) and elongated (0.1 × 0.5-1 μ) FC were found (Fig. 1f). A change of temperature did not affect the structure of FC of NRK and KNRK cells.

By the use of the new method, which has high resolution, it was possible to discover a new morphological variant of distribution of FC in minimally transformed NRK cells: small FC which, unlike contacts of cells of the primary fibroblast culture, fused together to form a dense peripheral network, were found in these cells; small contacts in the center of the cells were uniformly and singly arranged.

The network and separate contacts at the periphery of NRK and tsKNRK cells at the nonpermissive temperature were arranged in rows, repeating the outlines of the cells. These rows were perhaps formed at the border of the lamella [10]. During subsequent movement or spreading out of the cells in the same direction, rows of contacts that were immobile relative to the substrate [3] were found to be closer to the center of the cells, and new rows of contacts were formed along the border of the newly formed lamella.

The results of application of the new technique showed that FC are preserved in cells transformed by Kirsten sarcoma virus, although their shape, number, and arrangement were altered. Adhesion of transformed cells to the substrate thus takes place by basically the same method as adhesion of normal cells.

The main change undergone by FC in the cell system studied during transformation by Kirsten sarcoma virus is evidently a sharp decrease in their number: The shape of the contacts in some cases is unchanged.

The reduction in the number of FC may be connected with disturbance of the process of contact formation. Another possibility cannot be ruled out: during transformation of the cells the life span of the FC may be shortened. This could explain, first, the very small number or absence of "long-living" FC in the center of the cells and, second, the presence of only small contacts in KNRK cells. Contacts were perhaps formed but disappeared before attaining the size of a mature contact.

LITERATURE CITED

1. Yu. M. Vasil'ev and I. M. Gel'fand, Interaction of Normal and Neoplastic Cells with the Medium [in Russian], Moscow (1981), pp. 51-67 and 91-97.
2. A. A. Aaronson and C. A. Weaver, J. Gen. Virol., 13, 245 (1971).
3. M. Abercrombie and G. A. Dunn, Exp. Cell. Res., 92, 57 (1975).
4. M. Abercrombie, G. A. Dunn, and J. P. Heath, in: Cell and Tissue Interaction, ed. J. W. Lash and M. M. Burger, New York (1977), pp. 57-70.
5. M. Abercrombie, J. E. M. Heaysman, and S. M. Pegrum, Exp. Cell Res., 67, 299 (1971).
6. W. Birchmeier, Trends Biochem. Sci., 6, 234 (1981).
7. T. David-Pfeuty and S. J. Singer, Proc. Nat. Acad. Sci. USA, 77, 6687 (1980).
8. H. Duc-Nguyen, E. Rosenblum, and R. F. Zeigel, J. Bact., 92, 1133 (1966).
9. F. Grinell, Cell Biol. Int. Rep., 4, 1031 (1980).
10. C. S. Izzard and R. L. Lochner, J. Cell Sci., 21, 129 (1976).
11. A. A. Neifakh (A. A. Neyfakh), Jr., I. S. Tint, T. M. Svitkina, et al., Exp. Cell. Res., 149, 387 (1983).
12. A. A. Neifakh (A. A. Neyfakh), Jr., and T. M. Svitkina, Exp. Cell. Res., 149, 582 (1983).