TRANSPLANTABLE CELL LINE OBTAINED FROM RAT CARCINOMA

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As a result of prolonged cultivation of cells of a rat kidney carcinoma (strain RA) in a monolayer a new transplantable line of rat kidney carcinoma cells (RKC) was obtained, differing in its morphological and biological properties from the original.

Inoculation of cells of an RPK culture into rats led, in 1 of 18 cases, to the formation of a tumor capable of subsequent transplantation into animals.

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The few facts yet available to indicate that malignant cells, like cells derived from normal tissues, may undergo biological transformation during prolonged cultivation in vitro, as shown by the appearance of a transplantable cell line, are of particular interest to the understanding of the nature of malignancy [6, 8-14, 17-20].

The object of the investigation described below was to examine this phenomenon in the course of obtaining a transplantable cell line from rat carcinoma strain RA, which has so far received little study.

EXPERIMENTAL METHOD

The test object was a transplantable kidney carcinoma of solid structure of albino rats [1, 2], which had passed through more than 80 generations before these experiments began. According to our data [3, 4], the tumor possessed high virulence, especially for Wistar rats, and preserved its original morphological structure.

Primary monolayer cultures were obtained from this tumor by trypsinization [5]. Cells in a concentration of 5×10^5-10^6/ml were seeded into Carrel's flasks on medium No. 199 with the addition of a 30% solution of lactalbumin hydrolysate and of 20% bovine serum.

The cytologic study was undertaken on living cultures and also on stained preparations by the method described previously [7].

Growth of the cells cultures was studied quantitatively by determining the coefficient of proliferation and the percentage of dead cells, and measuring the pH of the medium in the course of cultivation.

EXPERIMENTAL RESULTS

Cells of the primary cultures of the tumor formed a monolayer on the 3rd-4th day, but showed low rates of growth (coefficient of proliferation in the first passages varied from 1.0-1.2). Most of the cultures died after 5-6 passages (2-3 months of cultivation) [3, 15].

Polymorphism of the cells was discovered by the cytomorphologic study of the primary cultures. The zone of growth consisted of a distinctive (loose in some places, condensed into structures resembling membranes in others) reticulum. In the membranes the cells were in contact with each other with no sharp lines of demarcation (see Fig. 1a).

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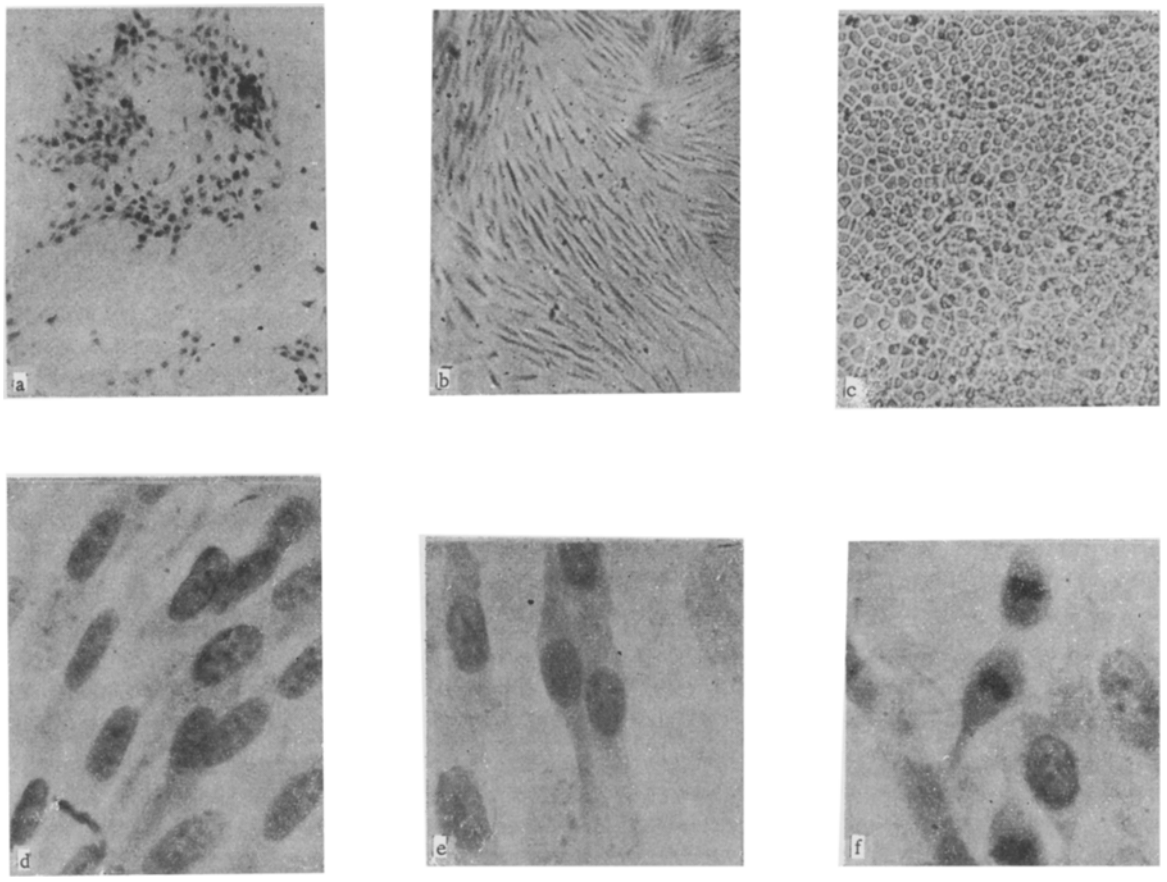

Fig. 1. Monolayer cell cultures of a rat carcinoma. a) Primary culture, 4th day from start of cultivation. Mayer's hematoxylin, 8 × 10; b) living culture, 4th month of cultivation. Fibroblast-like type of growth, 8 × 10; c) living culture, 8th month of cultivation. Polygonal cells in epithelial layer, 8 × 10; d) stretched cells, 11th month of cultivation. Mayer's hematoxylin, 90 × 10; e) spindle-shaped and flattened cells, 11th month of cultivation. Mayer's hematoxylin, 90 × 10; f) karyokinesis in a spindle cell, 11th month of cultivation. Mayer's hematoxylin, 90 × 10.

The looser areas consisted of spindle-shaped cells with interwoven processes. Their nuclei stained well and various phases of karyokinesis were found. In the later periods of cultivation the zone of growth was composed of elongated, spindle-shaped or irregular cells with several processes, with a tendency for predominance of a fibroblast-like type of growth. In some experiments the appearance of foci of round or epithelioid cells was observed against a background of a typically fibroblast-like type of growth, indicating that it is possible to obtain a transplantable cell line.

With this in mind, we carried out further experiments, but to provide less rigorous conditions for growth of the cultures they were not subcultured but maintained by frequent changes of nutrient medium. Observations continued for several months.

One of the Carrel's flasks in which a fibroblast-like type of growth was found after 3-4 months (see Fig. 1b) was kept under particularly close observation. The zone of growth of this culture consisted of intersecting streams of elongated cells all lying in the same direction. The nuclei of these cells were long and rod-shaped, or occasionally oval. None of the membrane-like condensations described above were observed in this reticulum.

In the 5th month a focus of cells differing sharply in shape from the surrounding fibroblast-like background was found in the zone of growth. The cells formed a layer thickened in the center. Its cells were oval, round, sometimes irregular, or angular and without processes. The periphery of this layer was loose and consisted of cells of the same oval, round, or irregular shape; in some places it was penetrated by spindle-shaped cells with processes. Compared with the original spindle-shaped type, these cells may be called granular.

Two weeks later two more such foci were found in the same flask. This suggested the multicentric, noncoincidental appearance of foci of cells differing morphologically from the cells of the initial zone of growth. Because of these changes and of the presence of living cells in the culture medium it was decided to pour off part of the old medium during one of its changes into fresh Carrel flasks. Fresh nutrient medium was added to these flasks, or in other words, the principle of conditioning of the medium [16, 21-23] was used. These subcultures by decanting were performed in the 5th-6th month every 5-10 days.

One month later growth of tiny colonies of cells was observed in the flasks with decanted medium, developing into epithelium-like membranes which later joined into continuous layers with loose borders; in the central parts of the layer, besides oval (granular) and round cells, polygonal cells appeared. By this time the fibroblast-like cells in most of the flasks had been replaced by epithelioid. Toward the end of the 6th and beginning of the 7th month, the number of polygonal cells in the membranes had increased.

Later, in order to obtain decanted subcultures, the flasks with the cultures were agitated gently, as a milder method of seeding than that employing the action of trypsin or versene. The daughter cultures showed fast rates of growth and further modifications in the shape of the colonies. The polygonal cells began to appear at the periphery of the colonies also. The edges of the colonies became wavy and had almost lost their looseness of structure. By the 8th month of cultivation the membranes were composed mainly of polygonal cells (see Fig. 1c), although here and there oval, angular, and sometimes irregular cells with short processes could still be seen.

Because of the increase in rate of growth of the subcultures, in the 9th month it was possible to change from conditioning to complete replacement of the medium and from shaking the culture to seeding with the aid of 0.25% trypsin solution. In the first subcultures an increasing number of fibroblast-like cells was found in the zone of growth, but polygonal cells were still present in the loose epithelial membranes. By the 10th month fibroblast-like cells were more numerous than polygonal cells. By the 11th month only a fibroblast-like zone of growth was observed, composed of bands of narrow cells lying in the same direction. These cells had basophilic cytoplasm and oval or rod-shaped nuclei, their long axis lying longitudinally in the cell; in which a few intensively stained nucleoli could be seen (See Fig. 1d). The ends of the cells were pointed or they branched into two or more processes, anastomosing with others. Besides spindle-shaped basophilic cells, a loose network of irregularly shaped, flattened cells with round, fleebly staining nuclei lay between the bands (see Fig. 1e). Karyokineses were often seen in the spindle-shaped cells (see Fig. 1f).

According to our data, the optimal dose for growth of the culture was a concentration of 30,000-50,000 cells/ml nutrient medium. The coefficient of proliferation on the 4th day reached 3.5 (in the primary cultures it did not exceed 1.2) and the pH of the medium was 6.6. Subcutaneous inoculation of Wistar rats with cells of 9-11-month old cultures (in a dose of 2×10^6-10^7 cells) led to the formation of a tumor in 1 of 18 cases, which was successfully inoculated into other Wistar rats. In the other 17 rats the tumors were absorbed.

At the present the cell line is in its 14th month of cultivation; seedings are carried out on the 5th-7th day of growth by means of trypsin and versene solutions. The culture has preserved the fibroblast-like type of growth as described above for 4 months.

The changes in the biological and morphological properties of the cells of these cultures thus show that a new transplantable cell line has been obtained. This lines has been called RKC (rat kidney carcinoma).

LITERATURE CITED

1. R. N. Akimova, Vrach. Delo, No. 6, 7 (1962).
2. R. K. Akimova, Vopr. Onkol., No. 12, 51 (1963).
3. Yu. T. Aleksanyan, In the book: Proceedings of a Conference of Junior Researchers at the Institute of Experimental Biology, AMN SSSR [in Russian], Moscow (1965), p. 3.
4. Yu. T. Aleksanyan, in the book: Proceedings of the 43rd Scientific Session to Review Work of Erevan Medical Institute [in Russian], Erevan (1966), p. 25.
5. O. G. Adzhaparidze, V. I. Gavrilov, B. F. Semenov, et al., Tissue Culture in Virologic Research [in Russian], Moscow (1962).
6. V. I. Gavrilov, Transplantable Cells in Virology [in Russian], Moscow (1964).
7. I. A. Glinskii, Byull. Eksp. Biol., No. 10, 84 (1964).
8. Ya. V. Dobrynin and R. P. Dirlygyan, Vopr. Onkol., No. 5, 46 (1961).

9. Ya. V. Dobrynin and R. P. Dirlygyan, Vopr. Onkol., No. 1, 74 (1961).
10. A. M. Eroshkina, Pat. Fiziol., No. 2, 23 (1959).
11. E. E. Pogosyants and A. F. Zakharov, In the book: Biology of Malignant Growth [in Russian], Moscow (1965), p. 152.
12. V. N. Ponomareva, Abstracts of Proceedings of the 4th Scientific Conference to Review Work of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1961), p. 62.
13. I. M. Surikov, In the book: Cytology of Malignant Growth [in Russian], Moscow-Leningrad (1963), p. 109.
14. A. D. Timofeevskii, Vopr. Onkol., No. 10, 3 (1960).
15. G. P. Tribulev, I. I. Podoplelov, Yu. T. Aleksanyan, et al., In the book: Proceedings of a Conference on Junior Researchers at the Institute of Experimental Biology, AMN SSSR [in Russian], Moscow (1966), p. 130.
16. U. Min, Abstracts of Proceedings of the 4th Scientific Conference to Review Work of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1961), p. 93.
17. G. Grey, W. Coffman, and M. Kubicek, Cancer Res., 12 264 (1952).
18. T. S. Hsu and O. J. Klatt, J. Nat. Cancer Inst., 22, 313 (1959).
19. A. Moore, L. Sabachewsky and H. Toolan, Cancer Res., 15, 598 (1955).
20. K. Norrby, O. Eriksson, and J. Mellgren, Ibid., 22, 147 (1962).
21. R. Parker, L. Castor, and E. McCulloch, In the book: Cellular Biology Nucleic Acids and Viruses, New York (1957), p. 303.
22. L. Sachs and D. Medina, Nature, 189, 457 (1961).
23. J. Westwood, J. Macpherson, and D. Titmus, Brit. J. Exp. Path., 38, 138 (1957).