PREPARATION OF HUMAN TUMOR STRAINS TRANSPLANTABLE IN NUDE MICE AND RATS FROM CELL LINES

E. S. Revazova, Yu. N. Solov'ev,
T. M. Khizhnyakova, and T. V. Yudicheva

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Many cell lines of human tumors transplantable *in vitro* already exist. Some of these lines, when transplanted into nude animals, have given rise to tumor strains. This paper describes six strains of human tumors, transplantable into nude mice and rats, in the collection of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, which were obtained from six cell lines forming part of the collection of D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

Nude mice based on line BALB/c, aged 6-8 weeks, and nude rats aged 4-6 weeks, reared by ourselves, were used. Mice received a subcutaneous injection of 10^6-10^7 cells from tissue culture in 0.5 ml of medium. Human hepatoma cell line Alex, containing genetic information of hepatitis B virus, synthesizes the surface antigen of that virus. This particular line, obtained from a primary hepatoma of the liver in 1976 [1], is growing in a monolayer. Cell line HT-29 of human colonic adenocarcinoma was obtained in 1975 [2] and is growing as a monolayer culture. Cell line Namalva, obtained in 1972 from a Burkitt's lymphoma, consists of lymphoblast cells of B type, and is cultured in suspension [4]. Cells of this line, in response to stimulation, produce large quantities of interferon. Another cell line of Burkitt's lymphoma (P3H3) was obtained from the ascites fluid of a patient in 1965 [5]. The line is growing in culture in suspension. Capsid and nuclear antigens of Epstein-Barr virus are always found in cells of this line, and 5-8% of the cells produce herpes-like virus particles. Cells of this line are used to produce Epstein-Barr virus. Cell line A-549 of human lung carcinoma was obtained in 1972 from tissue of a lung tumor [3] and is growing in a monolayer. It is highly sensitive to human adenoviruses. Cell line IJ of carcinoma of the urinary bladder was obtained from a bladder tumor [2]. The ras oncogene is constantly expressed in its cells, and it is growing in a monolayer.

The species to which the tumors of these transplantable strains belong was determined by electrophoresis of lactate dehydrogenase in agar gel.

EXPERIMENTAL RESULTS

Strain RPech of liver cancer, obtained from cell line Alex, is transplanted after 14-16 days. The strain consists of complexes separated by delicate connective-tissue bands. The cells are polymorphic, polygonal in shape, and closely packed together. Mitotic figures are numerous, PAS-positive material (glycogen) is present in the cytoplasm. In its structure the tumor corresponds to a picture of highly atypical hepatocellular carcinoma (Fig. 1a).

Cell culture IJ was the original material for strain RMP of carcinoma of the urinary bladder, transplantable at intervals of 10-11 days. This strain consists of transitional epithelium, a carcinoma of solid structure, with a tendency toward papilla formation (Fig. 1b).

Strain RTK-8 of colonic carcinoma arises from cell line NT-29 and is transplanted at intervals of 15-17 days. In the third subcultures strain RTK-8 was an undifferentiated adenocarcinoma with high mucus production (PAS-positive) (Fig. 1c). After 25 subcultures tumors

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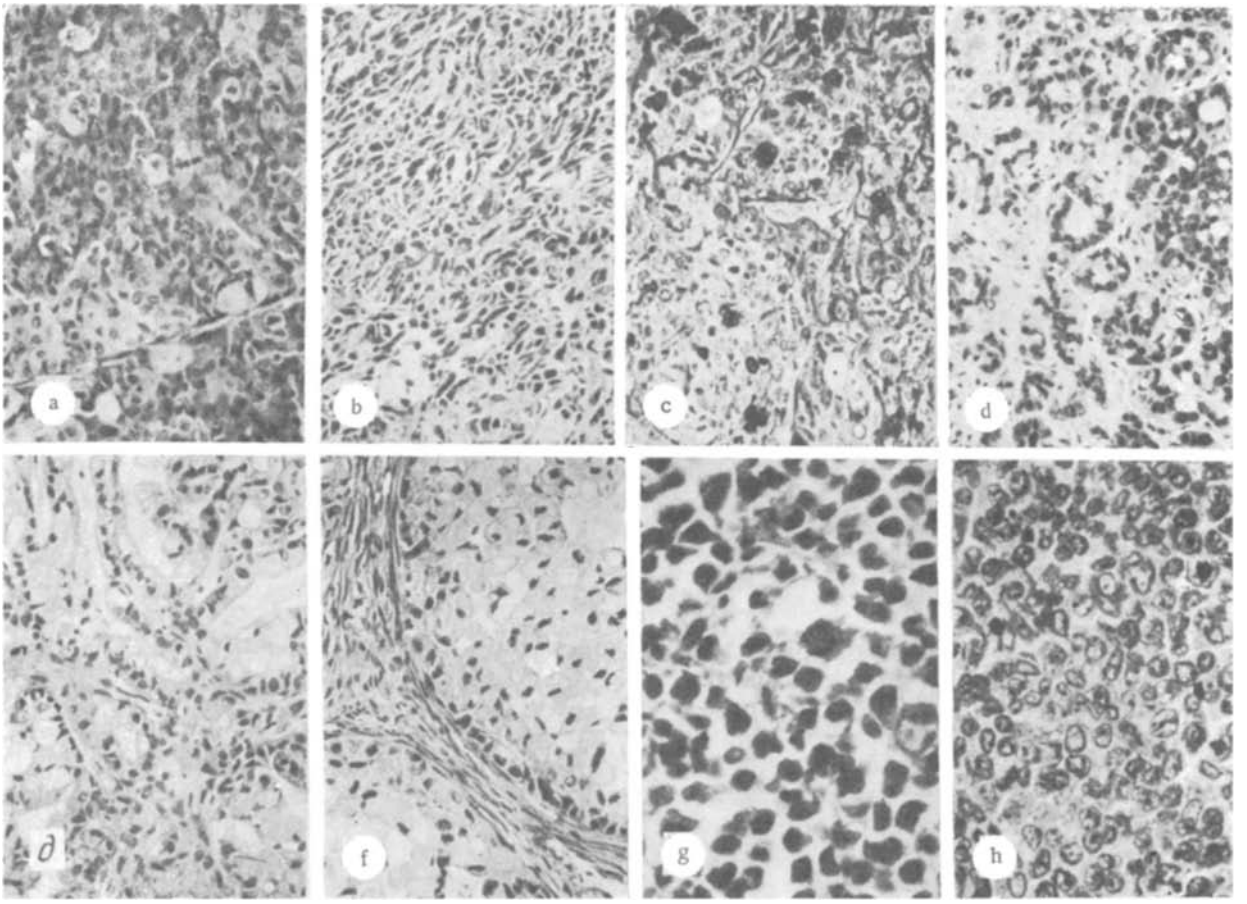

Fig. 1. Strains: a) RPech, 5th generation — highly atypical hepatocellular carcinoma, b) RMP (carcinoma of the bladder), 1st generation, transition-cell carcinoma; c) RTK-8 (carcinoma of the colon), 3rd generation, undifferentiated adenocarbinoma with high mucus production; d) RTK-9, 7th generation — moderately differentiated adenocarcinoma with mucus production; e) RL-4 (carcinoma of lung), 1st generation — adenocarcinoma with mucus production; f) RL-4, 1st generation, signet-cell carcinoma with mucus production; g) LB-P, 6th generation — Burkitt's lymphoma; h) LB-N, 2nd generation — Burkitt's lymphoma with high mitotic activity. a, b, d-h) Stained with hematoxylin and eosin; c) PAS reaction. Magnification: a-f) 160x, g, h) 400x.

of this strain were classified as a moderately differentiated adenocarcinoma with intracellular and intraglandular mucus production (Fig. 1d). Strain RL-4 of carcinoma of the lung was obtained from cell line A-549, and is transplanted from animal to animal after 32-35 days. In the first subcultures it was an adenocarcinoma with high mucus production (Fig. 1e, f). After serial transplantations into nude animals, it had the structure of a glandular-solid carcinoma with high mucus production.

Two transplantable strains of Burkitt's lymphoma LB-P and LB-N were obtained from different cell lines, namely P3H3 and Namalva respectively. Both strains were transplanted after 12-14 days and their histological structure was similar. Tumors of these strains consist of relatively monomorphic small cells with single large cells (Fig. 1g, h).

All six strains, transplantable into nude mice and rats, are human tumors. This is shown by the distribution of lactate dehydrogenase isozymes on electrophoresis in agar gel. Lactate dehydrogenase of all six strains had 5 peaks, a characteristic feature of human cells.

By serial passage in nude animals, strains of carcinoma of various human organs were thus obtained: the liver, colon, lung, urinary bladder, and also Burkitt's lymphomas. Despite the long duration (for several years) of transplantation of the cell lines *in vitro*, on transplantation of tumor cells in nude mice and rats tumors histologically identical with human tumors, from which cell lines were obtained, appeared in nude mice and rats. The strains obtained are

not only interesting for the experimental study of human tumors, but they can also be used for production purposes. For example, strain LB-N can produce human interferon, and strain LB-P can produce Epstein-Barr virus.

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CYTOTOXIC ACTION OF NATURAL KILLER CELLS ON HUMAN TUMOR CELLS

G. I. Avdeev, M. M. Vyadro, Yu. D. Leikin, E. A. Timofeevskaya, S. G. Osipov, and A. I. Semichastnyi

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An important role is nowadays ascribed in antitumor surveillance to cell-mediated natural cytotoxicity [3-5]. The principal property of natural killer cells (NKC) is their ability to exert a cytotoxic action on a broad spectrum of target cells, including malignant, virus-infected, as well as certain normal cells, spontaneously (without previous sensitization). In most investigations NKC activity has been detected on the basis of their action on leukemic and lymphomatous target cells. There have been very few studies of natural killer activity against cells of solid tumors [9-11].

Meanwhile data have been obtained on heterogeneity of the NKC population and the existence of NKC subpopulations both producing lysis mainly of lymphoma and leukemia cells (natural killer (NK) cells) and those having a cytotoxic action mainly on tumors cells of nonlymphoid origin (natural cytotoxic (NC) cells). In this connection the comparative study of the cytotoxic action of NKC on leukemia cells and on cells of human solid turmors is of great interest.

For this purpose the cytotoxic action of blood monocytes on cells of human tumor lines in culture was investigated in healthy donors: leukemic line K562 and line AKL of adenocarcinoma of the lung.

EXPERIMENTAL METHOD

Blood monocytes from healthy blood donors, obtained by centrifugation in a Ficoll-Verografin gradient and suspended in medium RPMI-1640 with 10% inactivated human serum (group IV), glutamine, and HEPES buffer (20 mM, complete medium RPMI-1640) in a concentration of 5×10^6 cells/ml, were used as effector cells. K562 and AKL cells were used as target cells. The K562 cells were grown in medium RPMI-1640 with the addition of 10% calf fetal serum, glutamine, and monomycin. Monolayer line AKL was grown in Eagle's medium with the additives mentioned above [2]. Target cells (2×10^6 cells in 1 ml of complete medium RPMI-1640) were incubated for 1 h at 37°C with 100 μCi of $Na_2{}^{51}CrO_4$ (USSR origin, specific radioactivity > 3 mCi/ml), after which the cells were washed 3 times and their concentration adjusted to 10^5 cells/ml with medium RPMI-1640. The cytotoxic test was carried out in plates for 16-18 h. To 0.1 of monocytes 0.1 ml of labeled target cells was added (ratio 50:1), and the mixture was centrifuged at 1000 rpm for 2 min. After the end of the reaction the plates were again centrifuged and radioactivity of 0.1 ml of supernatant determined on a "Gammacord" counter (Ames,

P. A. Gertsen Moscow Cancer Research Institute.

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