ACCUMULATION OF FLUORESCENT DYE ON NORMAL AND NEOPLASTIC CELLS

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In the diagnosis of tumors a fluorescence method based on selective accumulation of injected dyes is being used increasingly more often [3].

The disodium salt of fluorescein (FlNa), a xanthine dye with a high fluorescence emission, accumulates selectively in tumors and is nontoxic for the recipient.

Tissue culture provides the opportunity for undertaking fluorescence studies on relatively intact tumor cells and normal cells.

In the investigation described below, comparative accumulation of FlNa by cells of strains of various tumors and normal human fibroblasts, transplanted in culture, were studied.

EXPERIMENTAL METHOD

The material for the present investigation consisted of continuous cell lines of the following human tumors: carcinoma of the body of the uterus (CBU), rhabdomyosarcoma (RD), melanoma (MeWo), carcinoma of the urinary bladder (EJ), chorion-epithelioma (ChE), Wilm's tumor (WT), and osteosarcoma (HOS); postnatal human fibroblasts were used as normal cells. The karyotype of the cells corresponded to the original species.

The seeding dose was $(3\text{-}5)\cdot10^5$ cells in 1 ml. Cultures were grown in open flasks ($V = 50$ cm^3) in an atmosphere with 5% CO_2 at 37°C in Eagle's medium with 10-15% calf serum, 2 mM glutamine, and antibiotics. The conditions of isolation of the cultures were described previously [5].

Accumulation of FlNa in a concentration of 10^{-5} M in Hanks' solution by the cells was estimated on a spectrofluorometer at $\lambda_{ex} = 440$ nm and $\lambda_{em} = 511$ nm, by measuring the intensity of fluorescence.

Cells adherent to the glass were washed with Hanks' solution, covered with FlNa (2 ml), and the intensity of fluorescence of this solution (I_0) was measured and the sample incubated for 1 h at 37°C. The solution of FlNa was poured off after 1 h, the cells were washed with Hanks' solution to remove the excess of dye, and a fresh portion of Hanks' solution (2 ml) was added. The cells were again incubated for 1 h at 37°C to expel the accumulated dye from the cells. The intensity of fluorescence of the given solution (I_1) was then measured, the cells were removed from the glass with trypsin, and their number was counted.

The results of analysis were expressed in conventional units, using for the calculation the formula: $(I_1/I_0 \cdot N)\cdot100$, where I_0 denotes the intensity of fluorescence of a 10^{-5} solution of FlNa before incubation with the cells, I_1 the intensity of fluorescence of Hanks' solution with FlNa eluted from the cells, and N the number of cells (10^6/ml). The results were subjected to statistical analysis by nonparametric tests [2].

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TABLE 1. Accumulation of FlNa by Transplantable Tumor Cells and Normal Human Fibroblasts

Name of strain and cells	Postnatal fibroblasts
CBU	7,4
ChE	6,8
EJ	5,4
RD	4,8
MeNo	4,3
WT	3,34
HOS	2,26
Postnatal fibroblasts	1,2*

Legend. *) Differences from values for tumor cells are statistically significant.

EXPERIMENTAL RESULTS

Transplantation of transplantable cell lines into nude mice was accompanied by the development of tumors, whose morphological characteristics were identical with those of the original human tumors [4].

The serially transplanted diploid cell line of normal human fibroblasts had completed several passages in culture. On their transplantation into animals, no tumors developed. Altogether, more than 100 tests were carried out with cell cultures. Data on FlNa accumulation by the test cells are given in Table 1.

It will be clear from Table 1 that tumor cells differ significantly from normal cells in accumulation of the dye: the parameters of intensity of fluorescence for tumor cells lay within limits of 7.4 and 2.26 conventional units (c.u.), whereas for fibroblasts it was 1.2 c.u.

Tumor cells of varied genesis are characterized by their own individual parameters of FlNa accumulation. The most marked accumulation of the dye was observed for cells of strains CBU, ChE, and EJ (3-4 times higher than for fibroblasts).

Strain CBU was obtained from the human tumor strain CBU, transplanted into nude mice. This cell line has now passed through only 40 passages in tissue culture. A monolayer culture consists of epithelial and polygonal cells with large nuclei. On transplantation of the cells into nude mice, rapidly growing tumors corresponding in structure to undifferentiated carcinoma arise [5].

Strain EJ was obtained from a tumor of the human urinary bladder. The culture consists of epithelial-like cells with large nuclei. On transplantation into nude mice a rapidly growing tumor with the morphological picture of a transitional-cell carcinoma of solid structure is formed [7].

The greatest accumulation of fluorochrome was observed with tumor cells of strains WT and HOS.

Transplantable cell line WT was obtained from a strain of human tumor (nephroblastoma), transplanted into nude mice. The monolayer culture is polymorphic and consists of epithelial-like and fibroblast-like cells with a relatively wide (compared with the nucleus), vacuolated cytoplasm. On transplantation into animals a tumor corresponding morphologically to Wilms' tumor is slowly formed [5].

The HOS cell line was obtained from a human osteosarcoma. The culture consists of large polymorphic epithelial-like cells with large nuclei. Injection of the cells into animals induces a slowly growing tumor.

The lines of tumor cells transplanted in culture and strains of these same tumors transplanted into nude mice, as well as the above-mentioned properties, also have other characteristic differences. In particular, they induce a different response of the host animal to their transplantation, differ in their sensitivity to chemotherapy, and so on [1].

It is assumed that, by contrast with normal tissues, a particular feature of the morphogenesis of malignant tumors is the formation of pleiomorphic structures of low density from heterogeneous cells [6]. This feature is probably responsible for the definite interstitial characteristics of these structures during accumulation of dyes.

The different degree of FlNa accumulation by the test cells evidently characterizes their biological properties (rate of growth, morphological composition of the cells, character of their metabolism, and so on).

Karyotypic changes with an increase in ploidy characterize the degree of malignancy of tumor cells. Normal fibroblasts have a strictly diploid set of chromosomes. Strains CBU and EJ have a high content of a population of hyperdiploid cells with

a modal chromosome class of 60 and 88, respectively. The modal class of chromosomes for cells of strains HOS and WT, accumulating only a little dye, is 48 and 40, respectively.

Rapidly growing strains of tumor cells, forming large tumors on transplantation into animals, evidently accumulate more dye than cells of slowly growing tumors, containing a connective-tissue component, and normal fibroblasts.

The mechanism of selective accumulation of dyes by tumor cells has not yet been explained. Undoubtedly, the character of fluorochrome accumulation may be influenced by various exogenous and endogenous factors: changes in the pH of the surrounding medium, its ionic composition, the state of the transport system of the cells, metabolic additives, temperature conditions, adhesiveness of the cells, density of the cell monolayer, and so on. According to one report, if the gap junctions between membranes are disturbed, fluorescein is not transported between the cells [8].

A study of the particular features of cells of different tumors with the aid of fluorescent probes may probably prove useful for diagnosis, for assessment of the degree of malignancy, for determination of sensitivity to therapeutic agents, and investigation of the properties of the transport system of cells in vitro and in vivo.

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