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PREPARATION OF A STABLE RAT EMBRYONIC FIBROBLAST LINE BY TRANSFECTION WITH DNA FROM A PLASMID CONTAINING POLYOMA VIRUS LARGE T ANTIGEN

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KEY WORDS: immortalization; oncogenes; transfection.

Transfection of plasmids containing early genes of DNA-containing viruses (the large T antigen of polyoma and SV40 viruses, the EIA region of adenoviruses), and also genes of RNA-containing viruses (myc of virus MC29) into a primary culture of rat embryonic fibroblasts leads to changes in the properties of these cells, the most important of which is that the cells acquire capacity for unlimited division. In some cases dependence of the cells on serum factors is reduced in these lines and sensitivity to the transforming action of viral and activated cellular oncogenes is considerably increased [4, 7, 10, 11]. This last factor may be of great importance for the identification of transforming genes in human tumors by transfection of genetic material of tumor cells into recipient cells. Usually NIH3T3 cells, a culture of mouse embryonic fibroblasts, immortalized spontaneously, are used as recipients. By means of this system, activated oncogenes belonging chiefly to the ras family have been identified in many tumor cells [2]. These cells are perhaps permissive for manifestation of the transforming activity of this family of oncogenes. There is therefore an urgent need for the creation of new models appropriate for revealing the action of other oncogenes, in order to detect them in human and animal tumor cells. In particular, the REF-1 cell line has now been obtained after transfection of primary rat embryonic fibroblasts with DNA from the early region of simian adenovirus SA7 [1], which is used as the recipient system for transfection with oncogene-containing plasmid.

The aim of this investigation was to obtain an immortalized line of normal rat fibroblasts after transfection of a plasmid containing sequences coding the large T antigen of polyoma virus.

EXPERIMENTAL METHOD

Plasmid pPyLT1 was generously provided by Dr. Cuzin (France). It was constructed on the basis of plasmid pBR322 and contained an insert of sequences of the polyoma virus genome coding the large T antigen at BamH1 restriction sites [8].

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Fig. 1. Morphology of cell line REF (LT). Azure-eosin. 160×.

A primary culture of embryonic fibroblasts was obtained by trypsinization of 15-20-day old Fisher rat embryos. A cell culture at the second or third subpassage was used for transfection. The cells were seeded in a dose of $5 \cdot 10^5$ on 60-mm petri dishes in MEM medium (Serva or made in Czechoslovakia) with 10% embryonic calf serum, and 20-24 h later the cells were transfected with DNA of plasmid pPyLT1 by the calcium phosphate precipitate method [13]. A mixture of 8 μg plasmid DNA and 10 μg of high-molecular-weight salmon sperm DNA as the co-precipitant, after precipitation with calcium chloride, was applied on a 70% cell monolayer incubated for 4 h at 37°C in medium with 5% CO_2. The medium was replaced after incubation. The cells were kept on the dishes for 20 days, the medium being changed periodically. After 20 days the cells were trypsinized and subjected to long-term passage in the ratio of 1:3. Salmon sperm DNA was used as the control for transfection of the same cells. The efficiency of growth of the cells in semisolid agar was verified by determining colony-forming ability in 0.38% agar with 10% embryonic calf serum, the colonies being counted after two weeks [5]. The tumorigenicity of the cells was determined by transplanting them into nude mice aged 2 months in a dose of $(1\text{-}7) \cdot 10^6$ cells per animal.

EXPERIMENTAL RESULTS

To obtain a stable cell line primary rat embryonic fibroblasts were transfected with DNA of plasmid pPyLT1 containing the gene of the large T antigen of polyoma virus. After transfection no change in cell morphology was found and the cells were maintained on the same dishes for 20 days in medium with 5% embryonic calf serum. The cells were then trypsinized and subcultured. When the cells were seeded with low density (10^4 cells to a 10-cm petri dish), colonies of cells with the characteristic morphology of embryonic fibroblasts appeared on the dishes after 2 weeks. Colony formation after low-density passage of the cells is completely characteristic of immortalized cultures [12]. After passage of the control cells (primary rat embryonic fibroblasts, not transfected or transfected with salmon sperm DNA) with the same density (10^4 cells per 10-cm petri dish) colony formation was absent.

Unlike the control cells, which underwent degradation after the 6th-8th passage, cells after transfection with DNA of plasmid pPyLT1 were capable of unlimited division and went through more than 40 passages (more than 120 doublings), so that they could be regarded as immortalized [3], i.e., they turned into a line which was called REF (LT).

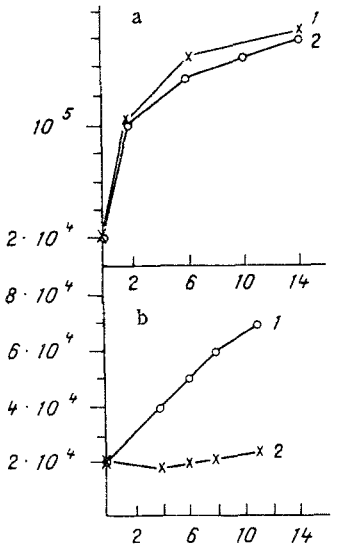

Fig. 2. Effect of serum concentration in medium on cell growth. a) 10% embryonic calf serum in medium: 1) cell line NIH3T3; 2) cell line REF (LT); b) 0.5% embryonic calf serum in medium. 1) cell line REF (LT); 2) cell line NIH3T3. Cells were subcultured in a dose of $2 \cdot 10^4$ on a 60-mm petri dish, and at definite time intervals the cells were trypsinized and counted. Abscissa, time of counting cells (in days); ordinate, number of cells in 1 ml.

REF (LT) cells have a fibroblast type of morphology, indistinguishable from the primary culture (Fig. 1). The growth properties of the REF (LT) cells in medium containing 10% embryonic calf serum were similar to stable untransformed cell lines such as NIH3T3. Cell division on a substrate was stopped after the formation of a monolayer with relatively low density, and these cultures all grew at the same rate (Fig. 2a). In semisolid agar (0.38%) REF (LT) cells, like NIH3T3, cells, did not divide and did not form colonies, i.e., their growth depended on the substrate. Similar properties, as was shown previously, also are possessed by the REF-1 cell line obtained after transfection of primary rat embryonic fibroblasts with DNA from the early region of simian adenovirus SA7 [1]. Unlike NIH3T3, however, REF (LT) cells could grow in medium containing only 0.5% embryonic calf serum (Fig. 2b), although the rate of growth was slower than in medium with 10% embryonic serum (Fig. 2a). This property, reduction of dependence of growth of the cells and serum factors, as was found previously is associated with expression of large T-antigen of polyoma virus [9].

Injection of REF (LT) cells into nude mice in a dose of $(1-7) \cdot 10^6$ per animal did not induce tumor formation in the mice even after a long time (3-4 months), i.e., the cells were nontumorigenic.

As a result of transfection of primary rat embryonic fibroblasts with plasmid DNA containing an insert of sequences of the large T antigen of polyoma virus, a stable cell line, namely REF (LT), was obtained. Monolayer growth on substrate, inability to grow in suspension, and absence of tumorigenicity indicate that this line is untransformed and can be compared with spontaneously immortalized lines of the 3T3 type [6]. At the same time, an essential difference between the REF (LT) line and lines of this type is reduced dependence of their growth on serum factors, due to the immortalization of these cells through the action of the gene of the large T antigen of polyoma virus [9]. Because of the properties of this REF (LT) line it can be used as recipient model with which to study the transforming action of activated oncogenes in human and animal tumor cells by the transfection method.

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DYNAMICS OF IMMUNOLOGIC AND CYTOCHEMICAL MARKERS DURING INDUCED DIFFERENTIATION OF CELL LINE K-562

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The process of differentiation of hematopoietic cells has been the subject of close attention by many investigators [1, 10], for knowledge of the laws of differentiation under normal conditions and their change during malignant transformation may provide a new approach to the treatment and diagnosis of various forms of leukemia and lymphosarcoma. Thanks to the production of monoclonal antibodies (MAB) to differential antigens, it has now become possible to make a detailed study of the process of cell maturation.

The aim of this investigation was to study changes in the immunologic phenotype of the K-562 cell line under the influence of the differentiation inducer tetradecanolphorbol-13-acetate (TPA) [1], by means of a panel of Soviet-produced antibodies, obtained at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

A panel of ICO MAB was used: ICO-1 against the monomorphic determinant of Ia-like antigens; ICO-02 against antigen of undifferentiated blast cells; ICO-10 against early thymocyte antigen (Thy-1); ICO-11 against function-associated antigen; ICO-GM1 against myelomonocytic antigen; ICO-12 against granulocytic antigen [2]. MAB of the HAE series, specific for erythroid differential markers also were used: HAE3 against glycophorin-A and HAE9 against erythroblast antigen [3]. Expression of the antigens was determined in the indirect immunofluorescence test (IFT), carried out in a cell suspension in plastic test tubes. The results of the IFT were read on a Leitz luminescence microscope, with magnification of 400×. Simultaneously preparations were made from the cell culture on a cytocentrifuge for morphological and cytochemical investigations. The morphological investigations were carried out after staining of films by Romanovsky's method, and the following enzymes were studied cytochemically: peroxidase, AS-D-chloroacetate esterase, α-naphthyl acetate, and butyrate esterase, with inhibition by sodium fluoride, acid phosphatase [4], and also siderophilic granules.

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