PRODUCTION OF TRANSFORMING GROWTH FACTOR BY CELLS OF A MOUSE SARCOMA INDUCED BY PLASTIC FILM

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So-called transforming growth factors (TGF) have recently been isolated from various neoplastic and some normal cells and tissues. Similar proteins have been found in the culture medium of cells transformed spontaneously or by oncogenic viruses and chemical carcinogens [7]. A distinguishing feature of TGF is their ability to stimulate growth of normal cells in a semisolid medium. Normal cells, as we know, cannot multiply in semisolid media, i.e., when not adherent to a substrate [1]. TGF reversibly transform normal cells, endowing them with the ability to multiply independently of the substrate, which is a feature of tumor cells [7]. TGF have not previously been isolated from tumors induced by the action of foreign bodies. Nevertheless, it can be postulated that proteins of this kind must play an important role in carcinogenesis of this type.

This paper describes an attempt to discover whether cells of a mouse sarcoma induced by subcutaneous implantation of polyvinyl chloride film can secrete a protein factor, phenotypically transforming normal cells.

EXPERIMENTAL METHOD

The following cell lines were used: PC-103 — a line of sarcoma cells maintained in culture and induced by subcutaneous implantation of polyvinyl chloride film into CBA mice [2], 2) NRK — a line of normal rat kidney fibroblasts [5]; 3) NIH 3T3 — a line of normal mouse fibroblasts. The cells were grown at 37°C: PC-103 on Eagle's medium with 10% bovine serum and monomycin (100 μg/ml); NIH 3T3 and NRK on medium RPMI-1640 with 10% embryonic calf serum and monomycin (100 μg/ml). The cells were used in the experiments after 5-26 passages *in vitro*.

The culture of PC-103 cells was washed 3 times, before forming a monolayer, with serum-free medium RPMI-1640. The first 24-h portion of serum-free medium was discarded, but the next two 48-h portions were used as conditioned medium (c-medium) [6]. To test its activity, the c-medium was centrifuged for 15 min at 2500 g and the supernatnat was filtered through millipore filters (pore diameter 0.22 μ).

For further purification of the c-medium it was centrifuged (200-250 ml) for 45 min at 100,000g. The supernatant was concentrated 20-25 times on an Amicon (USA) concentrator with IM-05 filter under nitrogen at a pressure of 2 atm. The concentrate was diluted with 1% acetic acid to the original volume and reconcentrated 20-25 times. This material was lyophilized and then extracted with 2 ml of 1 M acetic acid. The extract was centrifuged for 35 min at 100,000g. The supernatant was used for chromatography on a column with Bio-Gel P-60, equilibrated and eluted with 1 M acetic acid, uptake (A_{280}) was measured on a "Uvicord" instrument. The column was calibrated with proteins of known molecular weight. Fractions of 10 ml were collected from the column and freeze-dried. To test the activity of the freeze-dried material it was dissolved in serum-free RPMI medium (1-2 ml per fraction) and added to medium containing 1.2% methyl-cellulose. The c-medium at all stages of purification was kept at —70°C.

To determine the cloning efficiency in methylcellulose a 1.2% solution of methylcellulose (from Sigma, USA) in RPMI-1640 medium with 10% bovine (for PC-103) or embryonic calf (for NRK

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TABLE 1. Effect of c-Medium on PC-103 Cells on Multiplication of Various Cultures in Methylcellulose

Indicator cells	Mean number of colonies per dish in individual tests*	
	control	
	c.-medium not added	experiment
PC-103 (10^4 cells per dish)	0 0,2±0,22 0,75±0,29 1,25	22,6±5,08 19,0±4,99 9,2±3,74 14,75
NIH 3T3 (10^5 cells per dish)	2,5±0,58 0,8±0,65 0	54,3±2,48 50,8±3,15 30,0±14,0
NPK (10^5 cells per dish)	3,6±1,57 3,0±0,66	17,0±4,24 14,6±2,08

*Different lines in tables indicate results of different experiments.

TABLE 2. Effect of Conditioned Medium from NRK and NIH 3T3 Cells on Multiplication of Various Cultures in Methylcellulose

Indicator cells	Mean number of colonies per dish		
	c-medium —	c-medium NIH3T3	c-medium NRK
PC-103(10^4)	0,2±0,22	1,0±0,35	2,0±1,2
NIH 3T3(10^5)	0	0,2±0,22	—
	122,5±11,74	126,4±19,37	—
NRK ($5 \cdot 10^4$)	1,5±0,33	—	2

and NIH 3T3) serum was used. The c-medium was added in an amount equivalent to one-third of the volume of RPMI medium in the control methylcellulose. The concentrate after freeze-drying was added in an amount equivalent to the original c-medium, and fractions from the column were added in ten times that volume. The cells suspended in methylcellulose solution (2 ml per dish) were applied in a dose of 10^4 or 10^5 to the basal layer of 0.5% agar (Difco, USA), made up in culture medium with serum. For each experimental test 4 or 5 dishes were used. After incubation for 10-14 days in a moist atmosphere containing 5% CO_2 at 37°C the number of colonies was counted [4]. Regular round aggregates of cells at least 80 μ (PC-103) or 40 μ (NRK, NIH 3T3) in diameter were taken to be colonies. The diameter of the colonies was measured with an ocular micrometer, fitted into the objective of a "Diavert" microscope.

EXPERIMENTAL RESULTS

The c-medium from a culture of PC-103 cells was tested for its ability to induce the formation of colonies of NRK, NIH 3T3, and PC-103 cells in semisolid medium. The PC-103 cells, when seeded into medium with methylcellulose in a concentration of 10^4 cells per dish formed single colonies (cloning efficiency $0.2 \cdot 10^{-4}$ to $1.25 \cdot 10^{-4}$). The c-medium from PC-103 increased their number by more than an order of magnitude (Table 1).

NIH 3T3 cells formed single colonies on 10^5-10^6 cells applied to medium with methylcellulose. The c-medium from PC-103 stimulated the formation of NIH 3T3 colonies by about the same degree as colonies of PC-103 cells. The formation of NRK colonies in methylcellulose was stimulated rather less strongly by c-medium from PC-103 (Table 1).

The c-media from cultures of NRK and NIH 3T3 cells stimulated the formation of PC-103 colonies weakly, and did not stimulate the formation of colonies of the cells from which they were obtained (Table 2).

TABLE 3. Effect of Concentration, Partial Purification, and Freeze-Drying of Conditioned Medium PC-103 on Its Stimulating Activity

Indicator cells	Mean number of colonies per dish		
	c-medium	c-medium before concentration	c-medium after concentration and freeze-drying
PC-103(10^4)	0,67±0,5 1,25±0,86 7,0±2,4	7,4±0,67 9,0±2,45 —	13,6±2,41 — 170±15,38

After concentration, partial purification, and freeze-drying the stimulating activity of the c-medium was increased (Table 3). Heating to 60°C for 1 h did not change the activity of the c-medium.

Partially purified and concentrated c-medium from PC-103 was fractionated on a chromatographic column. Fractions collected from the column were freeze-dried from 1 M acetic acid and their growth-stimulating activity on PC-103 cells was tested. Fractions with mol. wt. of about 15 kilodaltons were found to have maximal activity.

The experiments thus showed that cells of a sarcoma PC-103 induced by subcutaneous implantation of chemically inert plastic film, like other transformed cells, produce a protein factor causing growth of a series of untransformed cells in medium with methylcellulose. The c-medium from cells of this culture significantly increase the number of colonies of PC-103 cells themselves in semisolid medium also. The growth-stimulating activity of the factor from PC-103 c-medium, its resistance to heat and acids, and also its low molecular weight, indicate similarity with the TGF isolated previously from other tumor cells. The ability of PC-103 cells to secrete TGF-like protein may play a role in the genesis of this tumor. Aseptic inflammation, terminating in encapsulation of the implant, arises at the site of introduction of a foreign body. It is evident that sarcomas induced by foreign bodies arise from local connective-tissue cells which participate in capsule formation [3]. Cells capable of secreting TGF, stimulating their own growth, under these conditions can proliferate preferentially. The limited space of the capsule, in the case of this type of carcinogenesis, may lead to accumulation of TGF and to a local increase in the concentration of these proteins.

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LITERATURE CITED

1. Yu. M. Vasil'ev and I. M. Gel'fand, Interaction of Normal and Neoplastic Cells with Medium [in Russian], Moscow (1981).
2. T. G. Moizhess, Tsitologiya, No. 2, 215 (1978).
3. T. G. Moizhess, in: Phenomena of Induction and Differentiation during Tumor Growth [in Russian], Moscow (1981), p. 99.
4. A. A. Stavrovskaya, T. P. Stromskaya, R. M. Brodskaya, and Yu. M. Vasil'ev, Genetika, No. 3, 434 (1982).
5. H. Duc-Nguen, E. N. Rosenblum, and R. F. Leiger, J. Bacteriol., 92, 1133 (1966).
6. G. E. De Larco and G. J. Todaro, J. Cell. Physiol., 94, 335 (1978).
7. A. B. Roberts, C. A. Frolik, M. A. Anzano, and M. B. Sporn, Fed. Proc., 42, 2621 (1983).