Selective Growth of Transformed Cell Lines by Rat Liver Perfusate

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SUMMARY

Perfused rat liver releases growth-promoting activity for viral, spontaneous, and chemically transformed cells. After 5 days of incubation with perfusate, cell lines 3T12-NY (a spontaneous fibroblast transformant), NQ-T-1 (a chemically transformed fibroblast line), W-8 (a chemically transformed epithelial rat liver cell line), and H-50 (an SV40-transformed hamster fibroblast line) all exhibit significant increase in cell growth above controls. Their respective normal counterparts: 3T3 CI 42, A31-714, K-16, and HEF are not so stimulated. Within another set, the virally transformed mouse fibroblast cell line, SV3T3, exhibits a 27-fold increase in growth; however, 3T3 (mouse fibroblasts), P3T3 (polyoma-transformed 3T3 cells), SV-F12-101 (a flat revertant line), and SV-P3T3 (a doubly transformed line) are nonresponsive.

Perfused rat liver also releases survival activity for SV-3T3 cells. The growth-stimulating activity in liver perfusate is selective for transformed cells. It is suggested that the liver may play a role in supporting neoplasia in vivo.

INTRODUCTION

Several experimental sources have shown that injections of rat liver homogenate can result in tumor induction (2, 11, 15) as well as increased tumor mass (9). Conversely, construction of a portacaval shunt leads to a decreased multiplication of SV40 virus-transformed mouse fibroblasts (Swiss 3T3) (8). To test the hypothesis that this transformation-specific growth enhancement is a general phenomenon, a comparison was made between the effect of TFGF on the growth of several transformed cell lines and its effect on their normal counterparts. Our results indicate that TFGF can support the growth of a variety of transformed cell lines in culture.

MATERIALS AND METHODS

Liver Perfusion. Male Sprague-Dawley rats weighing 115 to 150 g were fed Purina laboratory chow ad libitum. After anesthesia was induced with Nembutal (50 mg/kg), the livers were perfused as described previously (10). The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (7) containing bovine serum albumin (30 g/liter, Fraction V; Miles Laboratories, Inc., Elkhart, Ind.), 30% washed bovine erythrocytes (hematocrit of 25%), 15 mM glucose, and 10 times the normal plasma levels of amino acids (17). The perfusate was gassed with 95% O2:5% CO2 and maintained at 37°. After an initial 12-min washout, 400 ml of perfusate were recirculated through the liver at a flow rate of 7 ml/min. The perfusate was collected after 4 hr and centrifuged; the supernatant was then concentrated 10-fold by lyophilization prior to being assayed. Rat livers perfused under these conditions continued to synthesize protein at a constant rate for a period of several hr. TFGF accumulated in the perfusate at a linear rate throughout this period (8).

Cells and Growth Media. Swiss 3T3 and SV-3T3 cells were obtained from Dr. Marguerite Vogt and Dr. Renato Dubbeco. The cells were routinely cultured in Dubbeco and Vogt's modification of Eagle's medium in the presence of 10% FCS. The cells were transferred with the use of a Ca2+- and Mg2+-free 0.37% trypsin solution containing 0.14 M NaCl, 2 mM KCl, 4 mM Na2HPO4, 4 mM KH2PO4, and 5 mM dextrose. After centrifugation, the cells were washed twice in medium and plated at the appropriate density in 60-mm Falcon plastic dishes.

BALB 3T3 CI 42 and 3T12 NY cells were obtained from Dr. George Todaro. A31-714 and NQ-T-1 cells were obtained from Dr. J. DiPaola and Dr. T. Kakunaga. K16 and W8 cells were obtained from Dr. Bernard Weinstein. Primary HEF and H-50 cells were obtained from Dr. Fred Rapp.

Standard Assay for Growth Factor. Cells (104) of each cell line were plated on an amount of serum that did not induce a significant increase in cell number per dish over a period of 4 or 5 days. The CO2 concentration used was that which was described as optimum by the scientist who provided the cell line for these studies. 3T3 and SV-3T3 cells were plated in 0.4 and 0.15% FCS, respectively, and cultures were incubated in a 12% CO2 incubator. 3T3 CI 42 and 3T12-NY cells were plated in 0.4 and 0.25% FCS, respectively, and cultures were incubated in a 12% CO2 incubator. A31-714 and NQ-T-1 cells were plated in media containing 0.4 and 0.075% FCS, respectively, and cultures were incu-
bated in a 7% CO₂ incubator. K16 and W8 cells were cultured in media containing 0.6 and 0.2% FCS, respectively, in a 7% CO₂ incubator. Normal HEF cells were assayed as secondary cultures in Media 199 containing 10% typtose phosphate broth and 0.6% FCS; the H-50 cells were cultured in Eagle’s minimum essential medium containing 0.15% FCS in a 12% CO₂ incubator.

The sample to be tested was added (0.5 ml/5 ml medium). Numbers of cells were determined in a Coulter counter 5 days after the start of the experiment. All cell counts were performed in duplicate; all experiments were repeated at least twice.

**Standard Assay for Survival Factor.** SV-3T3 cells (10⁶) were washed 3 times by centrifugation in serum-free medium. The cells were plated in 5 ml Dulbecco and Vogt’s modification of Eagle’s medium in 60-mm Falcon plates without any added FCS. After 2 to 4 hr to allow the cells to attach to the dish, appropriate quantities of either rat serum or rat liver perfusate were added. Cultures were incubated at 37°C in a CO₂ incubator. Four days later, cell number was determined in a Coulter counter.

### RESULTS

The enhanced growth of SV-3T3 cells in the presence of liver perfusate is demonstrated in Chart 1. The flat revertant cell line, SV-F1-101, established by Pollack et al. (13) contains the SV40 viral genome, yet has a lower saturation density and tumor-producing capacity than SV3T3. The lack of growth stimulation in our assay indicated that the presence of the SV40 genome was not the prime determinant of the response of SV3T3 cells to TFGF. Polyoma virus-transformed mouse fibroblasts, P3T3, failed to respond to the liver perfusate. A transformed 3T3 cell line containing both the polyoma and SV40 viral genomes paralleled the response of P3T3, rather than SV3T3, when tested for the growth stimulation by TFGF. Normal Swiss 3T3 cells were unable to proliferate in low amounts of serum, and the addition of liver perfusate did not initiate growth.

Todaro and Green (16) showed that plating cells at a high density early in the establishment of a cell line resulted in one that exhibited an increased growth rate and a high saturation density, indicative of spontaneous transformation. Such a cell line is 3T12, derived from mouse embryo fibroblasts plated at 12 × 10⁵ cells/50-mm Petri (passed every 3rd day) until the development of the characteristics of an established line. This cell line was stimulated to grow by addition of TFGF; its normal counterpart, BALB CI 42, was not so affected (Chart 2).

Another means by which transformation can be induced is by incubation of cell cultures with chemical carcinogens. These cells, too, no longer grow in orderly fashion, have a decreased serum requirement, and result in tumors when injected into animals. Cell line NO-T-1, obtained by treatment of BALB 3T3 cells with 4-nitroquinoline 1-oxide, was stimulated to multiply upon addition of liver perfusate (Chart 2). Treatment of an epithelial rat liver cell line, K-16, with N-acetoxy-2-acetylaminofluorene produced trans-
formed cell line W-8 (18), which also demonstrated growth stimulation with TFGF (Chart 2).

Coincident with the fact that perfused hamster livers release a factor similar to rat liver TFGF in its ability to promote the growth of SV3T3 cells (J. Dietz and A. Lipton, unpublished observation), rat TFGF stimulated the growth of H-50 cells, a line obtained by SV40 virus infection of hamster embryo fibroblasts (Chart 2). No significant growth was observed in a secondary culture of normal hamster embryo fibroblasts within the 5-day period of incubation with the test samples.

It was also found that TFGF could stimulate the growth of 2 human cell lines. One of these, Raji, is a lymphoblast cell line derived from a Burkitt lymphoma and is presumably transformed by the Epstein-Barr virus (4). The 2nd is a human epithelial cell line, designated D-98 (5). This is a HeLa cell line, originally prepared from a human cervical tumor. However, there was observed only a 3-fold increase in cell number over a 7-day period in the latter cell line. This moderate response in the human cell lines to rat TFGF might be explained by the divergence of the species involved. No stimulation was observed in either human embryonic lung or human embryonic kidney cell cultures.

Most purified growth factors described to date do not allow for the survival of cells maintained in the absence of serum. In previous experiments, partially purified TFGF from serum did not contain any survival activity for SV3T3 cells (12). It thus became of interest to see whether liver perfusate could totally replace rat serum. As shown in Chart 3, rat liver perfusate allows for the survival and growth of SV3T3 cells when cells are plated in serum-free medium.

**DISCUSSION**

It has been reported that rat serum contains separate factors necessary for the growth of 3T3 and SV3T3 cells (12). Hepatoma cells have been shown to incorporate [3H]leucine and [3H]thymidine in response to a serum factor that has little effect on normal liver cells (3).

Pituitary and brain extracts have been shown to contain growth-promoting activity for 3T3 cells (1). The factor has been purified from bovine pituitary gland and has been called fibroblast growth factor (FGF). This polypeptide has a molecular weight of 13,300 ± 1,200. It stimulates DNA synthesis in 3T3 cells but does not cause the growth of SV-3T3 cells (6). Perfused rat liver releases a macromolecule that appears to differ from the pituitary factor. This TFGF appears to be a larger protein and promotes the growth of SV40-transformed 3T3 cells, but not that of 3T3 cells (8).

Various transformed cell lines can be stimulated to multiply in the presence of liver perfusate. In no case was buffer that had not passed through the liver responsible for an increase or a suppression of growth. Parallel samples of dialyzed liver perfusate demonstrate approximately equal activity as nondialyzed samples. Serum contamination was not responsible for magnitude of the observed responses as γ-globulin was not detectable in the concentrated perfusate (8).

Cells may be transformed by a variety of available techniques, i.e., by viruses, chemicals, and culture at high density (spontaneous). In this paper we show that a factor(s) present in liver perfusate can promote the multiplication of cells transformed by any of these techniques. Indeed, transformed fibroblasts, epithelial cells, and lymphoblasts can be induced to divide by a factor(s) present in the perfusate. The nontransformed cell is not so stimulated. It would appear that transformation by a variety of disparate methods results in a similar cellular alteration. Cells so affected can now be induced to grow in the presence of TFGF.

One possible explanation of these results is that the perfusate contains a growth inhibitory substance with a specificity for the nontransformed cells tested. When perfusate is added along with serum to nontransformed cells, no inhibition is noted. A 2nd possibility is that, because transformed cells in general respond to lower doses of serum than their normal counterparts, it would be possible to reproduce the growth curves shown by using a dose range of, for example, 0.1 to 2.0% calf serum. Such a result would not prove that serum was a specific factor for transformed cells, but only that the proper concentration for stimulation of normal cells had not yet been reached. Only further purification of the perfusate factor(s) will give a definite answer to this question. There would, however, appear to be no doubt that a factor(s) that promotes the growth of a variety of transformed cells is present in perfused rat and hamster liver. Due to the fact that separate factors appear to be limiting for the growth of 3T3 and SV3T3 cells, and the observation of Gospodarowicz (6) that FGF does not affect SV3T3 cells, we presume that the factor released from the liver is indeed a unique macromolecule.

On the basis of the above in vitro experiments, we feel that the liver merits further study for a determination of the extent to which it may be responsible for supporting neo-plastic growth in vivo.

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**REFERENCES**


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