Neoplastic Transformation of Hamster Astrocytes in vitro by Simian Virus 40 and Polyoma Virus

Abstract. *Astrocytes in cultures of brain cells from fetal or newborn hamsters undergo neoplastic transformation after infection with simian virus 40 or polyoma virus. Subcutaneous or intracerebral inoculation of the transformed brain cells into newborn or adult hamsters produces progressively enlarging astrocytomas at the sites of injection. Astrocytomas produced by polyoma-transformed cell lines are histologically better differentiated, but grow more rapidly and metastasize more frequently, than astrocytomas produced by cell lines transformed by simian virus 40. These observations make available in vitro models of virus-induced oncogenesis in astrocytes and provide simple techniques for obtaining astrocytoma cell lines suitable for screening studies of chemical agents effective against astrocytomas.*

An oncogenic DNA virus, simian virus 40 (SV40), has been shown to induce in vitro morphological transformations in human astrocytes (*1*) and in vitro neoplastic transformations in hamster neuroglial and meningeal cells (*2*). I report here that both SV40 and polyoma virus, another oncogenic DNA virus, can induce in vitro neoplastic transformations in cultured hamster astrocytes.

The leptomeninges of either fetal (⅔ gestation) or newborn Syrian hamsters were removed, and the brains from 12 litters of the former or 6 litters of the latter were pooled, minced, and trypsinized. Dispersed cell cultures of each type were established in test tubes and maintained as described (*2, 3*). On the day of preparation, ten cultures from the brains of newborns and ten from fetal brains were inoculated with $10^{6.5}$ $TCID_{50}$ (tissue culture infective dose, 50 percent effective) of SV40, and ten from fetal brains were inoculated with $10^{8.5}$ $TCID_{50}$ of polyoma virus. The remaining cultures (30 newborn and 20 fetal) were kept as virus-free controls. Derivation of the virus strains, methods of growth, and titration have been described (*2, 4*).

Cultures were observed daily for evidence of morphological transformation characterized by the emergence of colonies with an altered cellular morphology and accelerated multilayered growth. In all cultures infected with SV40, there was evidence of transformation within 50 to 70 days after inoculation. In those infected with polyoma virus, transformation was eventually observed in each culture, but was not recognized until 120 to 150 days after viral inoculation. There was no evidence of transformation in any of the uninoculated controls during 250 days of observation.

All of the SV40-transformed cultures and half of the uninoculated controls were trypsinized and subcultured 100 days after infection, and all of the polyoma-transformed cultures, as well as the remainder of the uninoculated cultures from fetal brains, were subcultured by the same method 170 days after infection. After this, all cultures were trypsinized and subcultured every 1 to 3 weeks, but only the transformed cultures survived after three to five passages. The SV40-transformed brain cells from nine cell lines of fetal origin and six cell lines from newborns were injected subcutaneously (10^6 cells from each line) into newborn hamsters 130 days after infection. Polyoma-transformed brain cells from four fetal cell lines were suspended and inoculated into newborn hamsters in an identical manner 210 days after infection. All of these animals developed neoplasms at the site of inoculation within 1 to 16 weeks. Animals inoculated with polyoma-transformed cell lines developed one or more palpable subcutaneous tumors earlier (1 to 3 weeks) than animals inoculated with cell lines transformed by SV40 (3 to 16 weeks). Once palpable, the neoplasms increased rapidly in size and, in every case, led to death of the host within 1 to 6 months.

In most instances, both the SV40- and polyoma-induced subcutaneous neoplasms remained encapsulated, but eventually they attained such enormous size and weight (up to 17 to 24 cm in diameter and weighing from 50 to 70 g) that the animals appeared to die of progressive cachexia. In a few cases the primary tumor was invasive of surrounding tissues, and the animals died as a result of direct extension of the tumor into vital structures. Multiple, discrete, small (pin-head to match-head size) tumor metastases were observed in lungs or kidneys, or both, but not in brain, in association with primary subcutaneous tumors induced by most (3 out of 4) of the polyoma-transformed cell lines, by some (2 out of 6) of the SV40-transformed cell lines from newborns, and by none (0 out of 9) of the SV40-transformed cell lines of fetal origin.

Microscopic examinations were performed on 153 of the primary neoplasms, including at least four induced by each of the transformed cell lines, and on each organ that exhibited macroscopically visible metastases. Tissues were fixed with Zenker's solution and embedded in paraffin; representative serial sections were stained alternately with hematoxylin and eosin or with phosphotungstic acid–hematoxylin.

Every primary and metastatic neoplasm induced by each cell line showed the histological characteristics of an astrocytoma (*5*) admixed with scattered small areas of choroid plexus papilloma (*6*). The histology of all tumors induced by a given cell line was remarkably uniform, but the histology of tumors induced by each of the three groups of transformed cell lines differed in characteristic ways. Thus, astrocytomas that developed in hamsters injected with SV40-transformed brain cells of fetal origin were histologically poorly differentiated (grade IV) and did not contain admixed areas of well-differentiated astrocytoma cells. In contrast, astrocytomas that developed in hamsters injected with polyoma-transformed fetal cells were histologically much better differentiated (grades I or II, or both) and did not contain undifferentiated (grades III or IV) areas. Astrocytomas that developed in hamsters injected with SV40-transformed brain cells from newborns were in some areas histologically well differentiated (grades I or II, or both) and in other areas histologically undifferentiated (grade IV). The SV40-induced astrocytomas produced by cultures of brain cells from newborns thereby corresponded to the classical characterization (*5*) of glioblastoma multiforme as comprising, histologically, both well-differentiated and poorly differentiated astrocytoma cells.

In addition to the subcutaneous inoculations of the transformed cell lines into newborn hamsters, three cell lines (one SV40-fetal, one SV40-newborn, and one polyoma-fetal) were inoculated intracerebrally (10^5 cells) into adult hamsters. In each case, neoplasms with the histological characteristics of astrocytomas arose at the site of inoculation within 2 to 6 months and eventually led to death of the host.

Astrocytomas arise by neoplastic alteration of astrocytes (*5*), but astrocytes are not found among subcutaneous tissues. Accordingly, the subcutaneous astrocytomas apparently arose by further multiplication in vivo of the inocu-

lated virus-transformed brain cells (that is, of virus-transformed astrocytes), and not by neoplastic transformation of subcutaneous cell types by infectious SV40 or polyoma virus associated with the cell inocula. Two additional kinds of evidence support this conclusion. Neither SV40 nor polyoma virus have produced tumors in newborn hamsters within 1 to 4 weeks; and we were unable to recover infectious SV40 from the SV40-transformed cells, even after growing the cells in direct contact with monolayers of primary cultures of African green-monkey kidney cells.

Our observations, therefore, demonstrate that cultured hamster astrocytes are susceptible to neoplastic transformation by both SV40 and polyoma virus. This susceptibility in vitro is in striking contrast to the apparent insusceptibility of astrocytes from newborn hamsters to neoplastic alteration induced by these viruses after intracerebral inoculation. Thus, after intracerebral inoculation into newborn hamsters, SV40 induces ependymomas (*7, 8*) and polyoma virus induces leptomeningeal sarcomas (*9*), but neither virus induces astrocytomas. The factors responsible for the different susceptibility of hamster astrocytes in vitro and in vivo remain undetermined. They may be related to the rapid rate of multiplication observed among astrocytes from newborn hamsters after explantation under the in vitro conditions of this study (*10*). Todaro and Green (*11*) have demonstrated that susceptibility to SV40-induced transformation in a rat cell line requires that the cells undergo at least one mitotic division after SV40 infection; mitotic figures have not been observed among astrocytes *in situ* in brains from newborn hamsters (*10*).

Both the tumor virus used to induce transformation and the developmental age of the cultured astrocytes employed for the transformation affected the degree of histological differentiation that was observed in the astrocytomas that resulted. The SV40-induced fetal astrocytomas were less well differentiated than SV40-induced newborn astrocytomas; and both groups of astrocytomas were less well differentiated than polyoma-induced fetal astrocytomas. However, these differences in histological differentiation were not associated with corresponding differences in the "malignancy" (that is, rate of growth, invasivity, and tendency to metastasize) of the astrocytomas. Thus, although histologically better differentiated, the polyoma-induced astrocytomas were at least as malignant as the histologically less well differentiated SV40-induced astrocytomas.

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References and Notes

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Increases in the Levels of Cytokinins in Bleeding Sap of Vitis vinifera L. after CCC Treatment

Abstract. *Paper-chromatogramed extracts of bleeding sap from grapevines treated with (2-chloroethyl)trimethylammonium chloride (CCC) contained higher levels of substances with cytokinin activity than did untreated plants. With the chromatographic solvent used, CCC was unlikely to interfere with the expression of the cytokinin response.*

Growth retardation by (2-chloroethyl)trimethylammonium chloride (CCC, Cycocel) is normally explained in terms of its effect on the biosynthesis of endogenous gibberellins (*1*). However, the stimulation by this substance of fruit set in *Vitis vinifera* (*2*) is not so readily interpreted on this basis, and it is evident that growth retardants exert other effects in addition to influencing gibberellin biosynthesis (*3*).

A previous report (*4*) described the morphological and cytological effects of CCC on roots of *Vitis vinifera,* and the prevention of these symptoms by gibberellic acid. Because CCC had such a marked effect on the size of the root tips (*4*), it seemed entirely possible that the levels of cytokinins in the bleeding sap (*5*) might be influenced by the treatment. This report describes results of cytokinin assays of bleeding sap from grapevines treated with CCC.

One-year-old plants of *Vitis vinifera* cv. Zante Currant (syn. Black Corinth), grown in a perlite-sand mixture in a greenhouse, were cut back to a single bud near the base of each plant, and when the new shoots from these buds reached a length of 30 cm, the plants were divided into two groups of 12 each. One group was treated three times weekly during April 1967 with 200 ml of a solution of CCC (1000 mg/liter), applied to the growing medium for 3 weeks, during which time the plants received Hoagland's solution once each week. At the termination of treatment, control shoots were 150 cm long, and treated shoots 100 cm. Sap was collected, chromatogramed, and assayed for cytokinin activity with soybean callus, as described in a previous report (*5*).

Figure 1 shows one series of assays from sap obtained between the 32nd and 56th hours after the commencement of collection; 130 ml of sap was collected from control plants and 152 ml from treated plants. In both cases

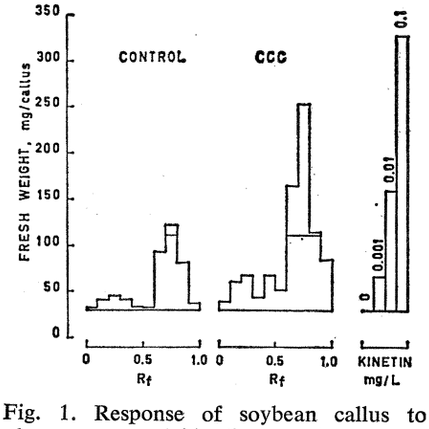

Fig. 1. Response of soybean callus to chromatogramed bleeding sap from Zante Currant vines. (Left) Sap from control plants. (Right) Sap from plants treated with CCC. The chromatographic solvent was a mixture of *n*-butanol, acetic acid, and water (4:1:1). Each assay was the mean of three replicate culture flasks. Calluses were grown at 27°C for 4 weeks. Statistically significant responses ($P = .05$) are shown above the upper horizontal line.