Cytoplasmic Suppression of Tumor Progression in Reconstituted Cells

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Abstract—This report details studies of whether mouse NIH/3T3 TG' karyoplasts that are exposed to benzo[a]pyrene epoxide(trans) (BPDE) can progress to tumorigenicity when they are rescued with either mouse B10mtJ CAP' tumorigenic (experiment 1) or nontumorigenic (experiment 2) cytoplasts. The mitochondrial DNA of the B10mtJ cells has restriction fragment length differences that allow distinction from the mitochondrial DNA of the NIH/3T3 cells. The reconstructed clones in experiment 1 were all tumorigenic, while those from experiment 2 were all nontumorigenic. The clones in both experiments were passaged for an equivalent time. These findings reflect the presence of factors in mouse cytoplasm capable of suppressing the tumor phenotype of NIH/3T3-BPDE treated karyoplasts when rescued at an early stage of progression.

INTRODUCTION

Numerous attempts have been made to repress cellular tumorigenicity by fusing to various types of tumor cells cytoplasts of enucleated nontumorigenic cells, i.e., through cybrid construction. The results of such experiments have been inconsistent in that no suppression (1), partial suppression (2-4), or virtually complete suppression (3) of cybrid tumorigenicity have been reported. We (5) and others (6) have discussed several reasons for the varying results. Israel and Schaeffer (6) found they could obtain virtually complete suppression of tumorigenicity by using the same cell line of tumorigenic and nontumorigenic rat liver epithelial cells, not mutagenizing the cells to introduce genetic markers, and using reconstructed cells (recons) made by fusing nontumorigenic cytoplasts to tumorigenic karyoplasts. By adhering to these three conditions, we could suppress tumorigenicity of recons made by crossing tumorigenic NIH/3T3 karyoplasts (k) to nontumorigenic NIH/3T3 cytoplasts (c) using a ricin-antiricin selection system (5). The NIH/3T3 cells had been incubated with benzo[a]pyrene epoxide(trans), BPDE, and passaged for several weeks before they became tumorigenic. This is in accord with the literature on chemical carcinogenesis (7-9). Cells after exposure to carcinogen and prior to expressing the tumor phenotype are referred to as being in a state of progression; they are potentially tumorigenic. We now report experiments in which we sought to suppress the eventual expression of tumorigenicity of recons in a very early stage of progression.

Karyoplasts from NIH/3T3 TG' nontumorigenic cells were incubated with BPDE and immediately rescued with cytoplasts obtained from either nontumorigenic or tu-
morigenic mouse B10mtJ CAPr cells which were chosen as the cytoplasmic donor since their mitochondrial DNA restriction patterns differ from those of NIH/3T3 cells thus allowing unambiguous proof that the reconstructed cells received B10mtJ cytoplasm. After selection in thioguanine and chloramphenicol, clones from both experiments were injected separately into nude mice with opposite results: In the first instance no tumors appeared in the nude mice; in the second, all the animals developed tumors. These experiments, like those of Israel and Schaeffer (6) point to the utility of using recons rather than cybrids in studies of cytoplasmic suppression of tumorigenicity and indicate that long-lived factors in nontumorigenic cytoplasm can block BPDE-treated karyoplasts from expressing the tumor phenotype.

MATERIALS AND METHODS

Carcinogen. The BPDE was obtained from the Midwest Research Institute and dissolved in DMSO that had been stored several months over the drying agent Aquacide I.

Animal Cells. Immortal NIH/3T3 Swiss mouse cells were obtained from Dr. Robert Weinberg. These cells originally became immortal spontaneously rather than by mutagenesis (10). The cells were cultivated in 1:1 (v/v) DMEM and Ham’s F12 containing 10% fetal calf serum, 1.98 mM L-glutamine, and 2.8 mM glucose. The cells were never allowed to become confluent since this potentially induces spontaneous transfectants. Cells resistant to 10 µg/ml 6-thioguanine (TG) were isolated by weekly passaging in increasing concentrations of the thio base. A second mouse cell type used for the construction of heterologous recons was called B10mtJ (11). It has the important characteristic of having a genetically identifiable mitochondrial genome. Mouse mitochondrial DNA of almost all inbred strains including C57BL/10 are identical. Mouse strain ddY (a wild strain found in Japan) has a unique type of mitochondrial DNA that can be distinguished from the old inbred strain by restriction fragment length differences (see Fig. 1). The following procedure was used to obtain the congenic B10mtJ mouse strain: The F1 females from the cross of ddY females and C57BL/10 males were back-crossed to C57BL/10 males, and the back-cross to C57BL/10 males was repeated for 20 generations. At this time the nuclear genome was shown to consist of C57BL/10 genes by examination of 20 biochemical chromosome markers, while the mitochondrial genome was the same as that of ddY (11). B10mtJ cells were isolated by culturing explants from 15- to 17-day-old fetuses. Similar to most rodent cells, the B10mtJ primary cells became immortalized spontaneously but were not tumorigenic in nude or B10mtJ mice. We have not obtained spontaneous tumorigenic cell lines from these immortalized cells, but treatment with BPDE did result in tumorigenic clones which were then used in control experiments. The immortalized B10mtJ cells were exposed to a weekly stepwise increase in chloramphenicol (CAP) concentration and became resistant to 100 µg/ml. This CAP resistance is most likely due to a mutation of the mitochondrial genome in the large ribosomal RNA gene and has been widely used as a dominant selectable cytoplasmic genetic mutation, since only cells that have this mutation will grow in the presence of CAP (12). Use of B10mtJ cells has the advantage that their mtDNA can be distinguished from that of NIH/3T3 cells by restriction fragment differences.

Heterologous Reconstructs. NIH/3T3 TGr cells were enucleated in Falcon T-25 flasks in 5 µg/ml of cytochalasin B with 85% efficiency (7500 rpm GSA rotor, 37°C, 30 min). The karyoplasts and a few whole cells that detached during centrifugation were subjected to a differential adhesion for 1.5 h to remove whole cells and karyoplasts containing large amounts of cytoplasm. The purified nonattached karyoplasts were then exposed to 0.5 µg/ml of BPDE for 30 min. Based on