ESTABLISHED CELL LINES FROM NONMAMMALIAN VERTEBRATES: MODELS FOR DNA REPAIR STUDIES

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SUMMARY

Several established cell lines from different classes of vertebrates were assayed for the presence of O'-methylguanine acceptor protein. This protein is instrumental in removing adducts from DNA caused by exposure to alkylating agents. Cultured cells had levels of acceptor protein activity within the range found in fresh tissues from animals in the same class. We suggest that cells from lower vertebrates are satisfactory in vitro models for studies of this DNA repair function.

Key words: DNA repair; nonmammalian vertebrates; models; cell lines.

INTRODUCTION

The use of cultured mammalian cells as models for studies of carcinogenesis and mutagenesis has been a highly productive undertaking (4). Mammalian cells in culture retain many of their in vivo functions; their response to treatment with carcinogens and mutagens can be correlated qualitatively with the response of cells in vivo, although quantitative predictions may not always be entirely accurate (3,5). Now interest is turning to the use of nonmammalian animal models in bioassays for evaluating environmental pollutants, particularly those occurring in natural waters. There is interest also in understanding the etiology of neoplasms in ectothermic vertebrates. Our concern was whether established cell lines from birds and ectotherms, especially fish, are as useful in predicting the cellular responses of intact animals as are mammalian cells for their animal counterparts. In particular, how far DNA repair capabilities were retained after ectothermic cells had been in culture for many passages or had been stored frozen.

Animals have a variety of mechanisms that protect them from damage to DNA caused by chemical and physical agents in the environment and by endogenous reactions. Defects in DNA repair have been implicated in malignant transformation and in normal and premature aging (10). Our work has centered on the ability of cells to remove adducts from their DNA caused by exposure to alkylating agents, such as N-nitrosamines. Such exposures lead to the formation of several adducts, including O'-alkylguanine, that may be responsible for the subsequent development of tumors (8). Cells remove this adduct by stoichiometric transfer of the alkyl group to the cysteine residue of an acceptor protein. Setlow et al. (7) and Waldstein and her colleagues (11,12) developed a simple assay for O'-methylguanine acceptor protein in extracts of cells from fresh tissue and from cultures; the assay is highly reproducible. We assayed acceptor protein activity in several established cell lines from the American Type Culture Collection and in a further three characterized cell lines from fish, and compared these values to our findings from a contemporaneous study of fresh tissue taken from animals of the same class (14). We comment also on the ability of fish cells in culture to photoreactivate cyclobutane pyrimidine dimers in DNA caused by exposure to ultraviolet radiation.

MATERIALS AND METHODS

Assay for acceptor protein activity. Details of the assay procedure have been given previously (7,14). Briefly, fresh tissues were collected from animals immediately after death and washed in homogenizing buffer consisting of 10 mM Tris-HCl; 1 mM EDTA; 1 mM DL-Dithiothreitol and 5% glycerol. The tissue was homogenized in ice-cold buffer and sonicated three times in a microultrasonic disrupter, centrifuged, and the supernatant used as the protein source. For assays with cells in culture, we used between two and three million cells that were obtained from one 75-cm flask in which the cells had almost reached confluence. The cells were removed by gently scraping the sides of the flask with a rubber policeman and then they were washed in phosphate buffered saline. The cells were centrifuged, transferred to the homogenizing buffer, sonicated three times in a microultrasonic disrupter, centrifuged, and the supernatant used as the protein source. For assays with cells in culture, we used between two and three million cells that were obtained from one 75-cm flask in which the cells had almost reached confluence. The cells were removed by gently scraping the sides of the flask with a rubber policeman and then they were washed in phosphate buffered saline. The cells were centrifuged, transferred to the homogenizing buffer, sonicated for 10 s, centrifuged again, and the supernatant used as the protein source. Thereafter, the procedure followed was identical for cultured cells and fresh tissue.

To each tube of protein extract was added exogenous radioactive calf thymus DNA labeled with tritium in the methyl group of the O'-methylguanine (1 pCi/mmol). The tubes were incubated at 37°C for 30 min allow transfer of
the labeled methyl group from DNA to the receptor protein. The reaction was terminated by the addition of 12 μl M HCl and incubation continued at 75° C for a further hour. The pellet was dissolved in 25 μl of 99% formic acid and reprecipitated with 150 μl 5% trichloroacetic acid; this step was repeated again. Finally the pellet was dissolved in a mixture of 100 μl formic acid, 1 ml of water, and 10 ml liquidscint, and radioactivity measured in a scintillation counter. The amount of 3H transferred to the acceptor protein was represented by the amount of radioactivity in the protein pellet. We measured the DNA content of the material by spectrofluorometry and expressed acceptor protein activity as femtomoles per microgram of DNA (2). All assays were repeated twice or three times.

Animals

All the animals used were kept in our animal facilities for a week before the assay to ensure that they were healthy and free from disease. Samples of tissue were taken from the liver, brain, kidney, and spleen.

Birds. Tissues were taken from fowls Gallus domesticus, from ducks Anas bochas, and from pigeons Columbus livia.

Reptiles. We used three species, the curly tailed lizard Anolis carolinensis, the spotted ameiva lizard Ameiva ameica, and the red ear turtle Pseudemys scripta elegans.

Amphibians. The assay was made on wood frogs Rana sylvatica, tree frogs Hyla cinerea, leopard frogs Rana pipiens, and firebelly toads Bombina orientialis.

Fishes. Assays included flounders Pseudopleuronectes americanus, Amazon mollies Poecilia formosa, and goldfish Carassius auratus.

Established Cell Lines. Cell lines obtained from a bird, a reptile, an amphibian, and from one species of fish were obtained from the American Type Culture Collection, Rockville, MD.

An embryo cell line derived from the Peking duck Anas platyrhyncus domesticus was assayed at the 8th passage; a cell line from heart tissue of a turtle (Terrapene) Terrapene carolina was assayed at Passage 101; bullfrog Rana catesbeiana cells, derived from tongue tissue, were assayed at their 98th passage; and acceptor protein activity of fathead minnow Pimephales promelas was assessed at Passage 78. The culture originally was established from tissue posterior to the anus.

Gem 218 and 81. These cell lines established in 1980 from spontaneous erythrophoromas of goldfish Carassius auratus were kindly donated to us by Jiro Matsumoto (Cancer Institute, Tokyo, Japan); GEM 218 was at Passage 6 and GEM 81 at Passage 37.

RBCF-I. This fibroblast line was established by Akihiro Shima (University of Tokyo, Tokyo, Japan) in 1980 from the caudal fin of a goldfish. We assayed the cells at Passage 35.

The American Type Culture Collection had cultured the bullfrog cells in minimal essential medium (MEM) with 10% fetal bovine serum (FBS) without antibiotics. We obtained much better growth of all three cell lines in Leibovitz 15 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin; this medium was diluted 20% for all cultures. The poikilothermic cells were kept at 25° C in an incubator without CO2. Duck cells were grown at 37° C in Dulbecco's modified medium with 10% FBS and antibiotics in an incubator with 7.5% CO2. The cell lines obtained from the American Type Culture Collection were free from mycoplasma, bacterial, and fungal contamination. Tests for mycoplasmal contamination in the other cell lines were negative (as determined by DNA staining with the Hoechst stain).

RESULTS

0'-Methylguanine acceptor activity

The values for the acceptor activity in fresh tissues and cultured cells are given in Table 1. For birds, amphibians, and fish, including the GEM lines established from tumors, the amount of activity in the cultured cells was of the same or higher than the range of activity found in fresh tissues. Acceptance protein activity in turtle cells both from fresh tissue and from cultures fell below that in fresh reptilian tissues; we cannot account for this difference.

DISCUSSION

By comparison with the number of mammalian cell lines available, there are rather few from birds and ectotherms. The American Type Culture Collection lists six Certified Cell Lines for birds, four for reptiles, and six each for amphibians and fish. Probably there are more in existence that have not been fully characterized and reported. Thus, Wolf and Mann (13) documented a total of 61 cell lines for fish, representing 17 families and 30 species. Some of these had been fully described, but most had not and were given only brief mention in the literature.

Recent years have seen a growth of interest in the use of nonmammalian models in bioassays and for cancer research. So far little mention has been made of the use of cultured cell lines from ectotherms, emphasis having been placed on the use of intact animals. We have shown here that the levels of acceptor protein activity are similar in cultured cells to those in fresh tissue, even after the cells have undergone many serial passages and after long periods in storage. For comparison, we assayed acceptor protein activity in two established mammalian cell lines and in four types of fresh tissue, from hamsters, sheep, and mice. Again, for this Class the activity in cultured cells is as high as or higher than in fresh tissue (Table 2). The higher levels of acceptor protein activity in cultured cells from mammals and from ectotherms were unexpected findings; we consider that the difference may be related to the mixture of different types of cells that occur in fresh tissue. The separation of hepatocytes from nonhepatocytes in liver, for example, may resolve this anomaly.

We have found that another DNA repair capability is retained in cultured cells from fish, that is, the ability to