ARYL HYDROCARBON HYDROXYLASE AND TYROSINE AMINOTRANSFERASE ACTIVITIES IN SOMATIC-CELL HYBRIDS DERIVED FROM HEPATOMA TISSUE CULTURE HTC (RAT) CELLS AND 3T3 (MOUSE) BENZO[a]PYRENE-RESISTANT CELLS

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ABSTRACT. Somatic-cell hybrids were formed between a 3T3 (mouse) benzo[a]-pyrene-resistant subline having very low basal or inducible aryl hydrocarbon hydroxylase and tyrosine aminotransferase activities and hepatoma tissue culture (rat) cells which lack the hydroxylase activity but contain the steroid-inducible aminotransferase. The benz[a]anthracene-inducible hydroxylase activity was absent or very low in all the hybrids. As has been the case in other hybrids from various parental lines, the aminotransferase was no longer inducible.

INTRODUCTION. Aryl hydrocarbon hydroxylase activity is inducible in mouse 3T3 fibroblasts by benz[a]anthracene (BA), whereas no detectable basal or induced level of this enzyme is detectable in rat hepatoma tissue culture (HTC) cells. Conversely, tyrosine aminotransferase activity is inducible in HTC cells by dexamethasone, whereas only low basal (i.e. not inducible) levels of this enzyme exist in 3T3 cells. In hybrids formed by fusion of these two parent lines (1), the level of BA-inducible hydroxylase activity ranged from that of the 3T3 parent (i.e. specific activity of about 3) to more than 10-fold greater. The hydroxylase specific activity in the majority of these BA-treated somatic-cell hybrids ranged between 6 and 40 (1). One question unanswered by these prior studies is which parental cell line is the source of the enhanced hydroxylase activity. Is the inherent hydroxylase of the 3T3 parent increased or is the potentially high hepatic hydroxylase of hepatoma cell origin being expressed?

In the present report, we have selected a 3T3 subline which is resistant to the toxic effects of 10 μg of benzo[a]pyrene per ml of growth medium. It has been established (2-4) that cells having the inducible hydroxylase activity demonstrate enhanced cytotoxicity, most likely because of epoxy and phenolic intermediates or products formed by the enzyme being induced. Exposure of 3T3 cells to increasing concentrations of benzo[a]pyrene in the growth medium over several weeks' time should therefore cause death of cells which have significant amounts of the hydroxylase and should allow the survival of cells that have little or no hydroxylase activity. As predicted, by means of this technique we developed a cell line which contained very low basal or inducible hydroxylase activity. We then studied fused-cell hybrids between the HTC parent and this 3T3 subline. These hybrids showed negligible or only small magnitudes of induction of both hydroxylase and tyrosine aminotransferase activities. These results therefore suggest that the hydroxylase in hybrids from our previous study (1) is of 3T3 origin and/or that the present 3T3 variant cells possess a dominant factor which prevents expression of hydroxylase activity.

MATERIALS AND METHODS

The enzyme assays for hydroxylase and aminotransferase activities, the protein determinations, and the chromosomal preparations were performed exactly as previously described (1). One unit of aryl hydrocarbon hydroxylase activity is defined as that amount of enzyme catalyzing per min at 37° the formation of hydroxylated product causing fluorescence equivalent to that of 1 pmole of 3-hydroxybenzo[a]pyrene. Hydroxylase specific activity is expressed as units per mg of cellular protein. Tyrosine aminotransferase specific activity is expressed as nmoles of p-hydroxyphenylpyruvic acid formed per min per mg of cellular protein. HMT medium is Eagle's culture medium (1) containing 100 μM hypoxanthine, 0.4 μM methotrexate, and 16 μM thymidine.

Cell lines. HTC (AR1, TAT^+, AHH^-) is a subline of HTC cells that was derived from wild-type HTC cells (5) and is resistant to 6-mercaptopurine at con-

centrations as high as 10^{-4}M. These cells lack hypoxanthine-guanine phsophoribosyltransferase and therefore cannot survive in HMT medium. This HTC subline possesses glucocorticoid-inducible tyrosine aminotransferase (TAT^+) but lacks aryl hydrocarbon hydroxylase activity (AHH^-).

The 3T3-4(E) clone is a thymidine kinase-deficient 3T3 line generously given to us by Dr. Howard Green, Massachusetts Institute of Technology, Cambridge (6). The 3T3 cells were grown in increasing concentrations of benzo[a]-pyrene in the growth medium: 0.1 µg/ml the first week, 0.5 µg/ml the second week, 1.0 µg/ml the third week, 5 µg/ml the fourth week, and 10 µg/ml thereafter. The most actively growing clone which showed no morphological signs of toxicity to continuous levels of 10 µg benzo[a]pyrene per ml was selected and designated the 3T3-4(E) (BP^R, TAT^-, AHH^-) subline: benzo[a]pyrene-resistant and both tyrosine aminotransferase and aryl hydrocarbon hydroxylase activities are very low or absent. These cells lack thymidine kinase and therefore survive in HMT medium We have also found (unpublished data) that cultures exposed to increasing concentrations of 7,12-dimethylbenz[a]anthracene will eventually develop clones having negligible basal and inducible aryl hydrocarbon hydroxylase activities.

Cell fusion. The fusion process, enhanced by addition of β-propiolactone-inactivated Sendai virus, and the selection of the hybrids were carried out by the methods previously described (1). After one month in selective medium, many putative hybrid colonies in one flask were treated with trypsin, and 200 cells each were plated in a series of tissue culture dishes. The dishes contained broken cover slips; it therefore was possible later to remove cover slip pieces which contained only a single colony. Each hybrid colony reported below was obtained from such a glass chip and therefore probably represents an independent hybridization event. The resultant clones were grown an additional month in HMT medium before enzyme and chromosomal analyses were performed.

RESULTS AND DISCUSSION

The 3T3-4(E) line had only telocentric chromosomes (1) and a narrow range of 68 to 69 (Table I). The 3T3-4(E) (BP^R, TAT^-, AHH^-) variant contained 59 to

Table I. Chromosomal complement and maximally inducible hydroxylase and aminotransferase activitities in the 3T3-4(E) line, the HTC (AR1, TAT^+, AHH^-) and 3T3-4(E) (BP^R, TAT^-, AHH^-) parent sublines, and 13 hybrid clones.

Cell line	Chromosomes		Specific enzyme activity: Aryl hydrocarbon hydroxylase		Specific enzyme activity: Tyrosine aminotransferase	
	Total range	percent Bi-armed	Basal	Benz[a]anthracene-treated	Basal	Dexamethasone-treated
3T3-4(E)	68-69	0	0.2	3.2	0.8	0.8
Parental sublines						
3T3-4(E)(BP^R, TAT^-, AHH^-)	59-69	0	0.2	0.3		
HTC (AR1, TAT^+, AHH^-)	58-129[a]	29-59[a]	<0.2[b]	<0.2	8.4	16.3
Hybrids						
Clone 10	110-137	24-31	<0.2	<0.2		
11	115-123	28-31	<0.2	<0.2		
12	97-109	29-34	<0.2	<0.2		
13	103-111	24-29	<0.2	<0.2		
15	90-101	22-24	<0.2	<0.2		
AB1	100-105	23-27	<0.2	0.3	0.8	<0.1[b]
AB2	98-109	24-27	<0.2	0.4	0.3	<0.1
AB3	119-129	26-29	<0.2	0.4	0.6	0.4
AB5	92-101	24-26	<0.2	<0.2	0.2	0.9
AB7	103-105	25-28	<0.2	0.2	1.4	1.4
AB8	101-112	26-29	0.2	0.4	1.4	0.1
AB4	95-111	23-26	<0.2	1.1	<0.1	<0.1
AB6	91-115	20-26	1.2	0.6	1.9	1.8
	57-59	12-19				

Footnotes to Table I

[a] Fifty percent of HTC (AR1, TAT^+, AHH^-) metaphases contained 58 to 67 chromosomes, and about 25% were clustered between 121 and 129. Approximately one-half of the chromosomes in each metaphase were bi-armed (cf. ref. 1).

[b] Below the limits of sensitivity for the enzyme assay.

69 chromosomes with no strong mode; of 40 metaphases counted, 32 ranged between 62 and 67. All chromosomes from this mouse-derived parent subline were telocentric. As noted before (1), the HTC line contains a modal number of about 62 chromosomes, about half of which are bi-armed. Hybrids were therefore determined by differential chromosome counts, by following a 3T3 marker chromosome (1), and by survival in the selective HMT medium. Cells of the parental lines 3T3-4(E) (BP^R, TAT^-, AHH^-) and HTC (AR1, TAT^+, AHH^-), in numbers equal to those used in the fusion experiments, were carried separately through a mock fusion process and then were grown in selective medium (1); none survived and the tissue culture plates were discarded after one month.

Chromosomal analysis was done on 5 to 10 metaphases each of 20 putative hybrids obtained as single colonies on cover slip chips. Thirteen hybrid colonies, identified on the basis of karyotype, were then examined in detail for inducible hydroxylase or aminotransferase activity. In 11 of the 13 hybrids, one to six days of continuous treatment of the cultures with 13 μM BA--the concentrations known to induce the hydroxylase activity to its fullest extent in 3T3-4(E) cells--failed to stimulate the enzyme activity. Two clones showed interesting differences from the rest. Clone AB4 showed a significant rise in BA-inducible hydroxylase, a result consistent with segregation and loss of some negative control element which is still present in the other clones. Clone AB6 had a bimodal distribution of chromosomes, with some cells consistent with a 1S + 1S hybrid partially segregated and with other cells showing extreme segregation. The basal hydroxylase activity in this clone was higher than that in either the original 3T3-4(E) cell line or the 3T3-4(E) (BP^R, TAT^-, AHH^-) parent subline, and the enzyme activity was unchanged or decreased slightly upon one to six days of exposure to BA.

In the 8 clones examined for tyrosine aminotransferase activity, the enzyme was uniformly low and not inducible by dexamethasone. This finding is consistent with results from several previous studies of fused-cell hybrids (1, 7-10). A recent study (11) involving mouse-mouse and human-hamster hybrids

demonstrated varying degrees of BA-induced hydroxylase activity that depended on the parental lines involved. In our previous report (1), 3T3-4(E)(mouse) x HTC(rat) hybrids showed a marked enhancement of BA-inducible hydroxylase (i.e. specific activity between 2.5 and 62) compared with a maximally induced specific activity of about 3.0 in the 3T3-4(E) parent. In the cross described in this paper, hybrids formed by HTC cells and a 3T3 variant--selected because it lacked the hydroxylase--failed to show this enhancement. We believe that these results reinforce the view that the enhancement of aryl hydrocarbon hydroxylase induction seen in 3T3-4(E) x HTC hybrids (1) is caused by an increase in 3T3-specific mRNA, protein, and hydroxylase activity. We cannot, however, rule out the possibility that the process by which the 3T3-4(E) (BP^R, TAT^-, AHH^-) variant was selected might have created some dominant factor which has the capacity to repress BA-inducible hydroxylase activity.

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