ANL/PPRNT--89-191

DE90 003004

Induction of Sister Chromatid Exchanges by Direct and Indirect Chemical Agents in a Human Teratoma Cell Line

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DISCLAIMER

Abstract

In the present work, we have extended the characterization of the P3 cell line, derived from a human epithelial teratocarcinoma, by studying the induction of sister chromatid exchanges (SCEs) by direct and indirect carcinogens. Several direct-acting carcinogens produce a dose-dependent increase in SCEs. Most notably, N-methyl-N'-nitro-N-nitrosoguanidine and 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene produce increases in SCEs at dosages comparable to those used to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase locus. The indirect carcinogens elicit SCEs only when the P3 cells are cocultured with cells capable of metabolizing the indirect carcinogens to the active form. Human breast carcinoma (BJ-015) and rat hepatoma (RL12) cells are equally efficient in activating polycyclic aromatic hydrocarbons to the active form. This cell-mediated induction of SCEs is obtained when P3 cells are incubated with live, x-irradiated, or UV-irradiated BJ or RL cells. This P3 cell line is thus equally suitable to study the induction of mutations or the induction of SCEs with direct and indirect carcinogens.

Introduction

To screen the many chemicals that may prove to be genotoxic to man, the most useful assays are those involving human tissues or cells. These assays may clarify the nature of the processes that occur in the human body and may indeed contribute to the evaluation of the validity of extrapolating data obtained from animals to man.

Recently, several investigators have used fibroblast cell strains to study the mutagenic response to physical and chemical agents (Gupta and Siminovitch, 1978; Jacobs and DeMars, 1978; Glover et al., 1979; McCormick and Maher, 1981). Because the fibroblasts have a limited life span, grow slowly in culture, and generally lack the ability to metabolize indirect carcinogens to the active form, they are not generally used in screening assays. On the other hand, the lymphoid cell lines used by Thilley et al. (1980) in mutagenesis assays grow continuously in culture. In both cases, however, the cell strains and the cell lines will respond to indirect or procarcinogens only after these compounds are activated with the subcellular fractions of rat liver homogenates or when the cells are cocultivated with normal or tumor cells able to metabolize procarcinogens (Aus et al., 1980; Michalopoulos et al., 1981; Strom et al., 1981; Tong et al., 1981; Bhatt et al., 1983). Cocultivation of the test cells with live cells may, in fact, chemically activate the procarcinogens in a manner more closely resembling that observed *in vivo* (Newbold et al., 1979; Sebti et al., 1982; Diamond et al., 1980). Other studies have shown that cell-mediated mutagenesis assays may reflect cell/organ specificity and the *in vivo* carcinogenic potency of the tested chemicals (Gould, 1982; Huberman and Sachs, 1976; Huberman and Slaga, 1979; Jones and Huberman, 1980; Kuroki and Drevon, 1978; Lagenbach et al., 1978;

1981; Newbold et al., 1979; Raveh et al., 1982; Reiner et al., 1983). Human tumor cell lines capable of metabolizing procarcinogens have also been used directly to examine the genotoxic responses of the cells by measuring the induced increases in sister chromatid exchanges, SCEs (Abe et al., 1983; Huh et al., 1982; Dearfield et al., 1983). However, because these cell lines are generally aneuploid, they may not be as useful for mutagenesis assays.

A previous report described the use of a human epithelial teratocarcinoma cell line (designated P3) as a target cell in a mutagenesis study with direct and indirect carcinogens. The P3 clonal isolate was shown to have a stable diploid karyotype with 46(XX) chromosomes and a translocation between chromosomes 15 and 20. Mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus could be induced by polycyclic aromatic hydrocarbons (PAH) and by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (Huberman et al., 1984).

In the present work, we have used the P3 cell line to study the induction of sister chromatid exchanges by direct and indirect carcinogens. At least three different cell lines were used for metabolic activation of the indirect carcinogens. These results provide additional characterization of the P3 cells and demonstrate the usefulness of this cell line for the detection of direct and indirect mutagens by the induction of SCEs.

Materials and methods

Chemicals

Benzo(a)pyrene [B(a)P]; benzo(e)pyrene [B(e)P]; 7,12-dimethylbenz(a)anthracene (DMBA); dimethylnitrosamine (DMN); N-methyl-N'-nitro-N-nitrosoguanidine (MNNG); N-methylnitrosourea (MNU); Mitomycin C;

5-bromodeoxyuridine (5-BudR); 4-nitrosoquinoline-1-oxide (4-NQO); cyclopenteno(c,d)pyrene (CP); α-naphthoflavone (NF); Colcemid; methylcholanthrene (MCA); and 6-thioguanine (TG) were obtained from the Sigma Chemical Company, St. Louis, MO, the Aldrich Chemical Company, Milwaukee, WI, and from the National Cancer Institute, Bethesda, MD. All carcinogens were dissolved in dimethyl sulfoxide (DMSO) prior to their dilution into the culture medium. The 5-BudR, Mitomycin C, and Colcemid were dissolved in Dulbecco's phosphate buffer solution (PBS).

Cells

The characteristics of the P3 clonal isolate obtained from a human teratocarcinoma were previously described (Huberman et al., 1984). The breast epithelial cell line BJ-015 derived from a human breast carcinoma was obtained from the Naval Bioscience Laboratory, Oakland, CA. The rat hepatoma cell line RL-12 was previously shown to metabolize a number of PAH (Murison et al., 1984). All cell lines were routinely cultured in RPMI-1640 medium supplemented with 20% fetal calf serum with penicillin (100 units/mL) and streptomycin (100 µg/mL), Gibco, Grand Island, NY.

Sister Chromatid Exchange Analysis

Direct-Acting Carcinogens. The P3 cells were subcultured with 0.05% trypsin and 0.02% EDTA (Gibco) and transferred to 60-mm dishes at 2×10^5 cells per dish. After 12-24 h, the cells were treated with the desired concentration of carcinogen dissolved in DMSO to a final concentration of DMSO not exceeding 0.05%. After 4-12 h of exposure, the medium was removed and fresh culture medium was added with 10 µg/mL of 5-BudR. The cells were grown in the dark for at least 2 cell cycles (approximately 36 h). Colcemid

was added to 0.05 μg/mL for the last 2 h of incubation. The cells were harvested, suspended in hypotonic KCl (0.075M), and kept at 37°C for 10 min. The cells were then fixed in at least three changes of glacial acetic acid:methanol solution (1:3), and the cell suspension was dropped onto clean glass slides. Two-day-old slides were then processed for SCE analysis by using a modification of the hot salt Giemsa technique (Korenberg and Freedlender, 1974).

Indirect Acting Carcinogens. The culture dishes were first seeded with 1 x 10^6 BJ-015 cells irradiated with either x-rays at 5000 rad or ultraviolet light at 40 J/m^2 to inhibit cell division. The next day, the irradiated cells were overlaid with 3 x 10^5 P3 cells in 4 mL of fresh growth medium. Four hours later, the cells were exposed to the test procarcinogen dissolved in DMSO. The cell-mediated activation was allowed to proceed for 24-48 h, after which the cells were subcultured with trypsin:EDTA (3:1) in fresh growth medium containing 10 μg/mL 5-BudR. After two rounds of cell division in the dark, the cultures were once more harvested for SCE analysis as described above.

Hydrocarbon-Metabolizing Activity Assay

The hydrocarbon-metabolizing activity of the cell lines was determined by measuring the ability of the cells to convert tritium-labeled B(a)P to the aqueous soluble form by using the method of Huberman et al. (1971). Cells seeded at 1 x 10^6 into 75 cm^2 flasks were incubated with 5 μCi/mL H^3-B(a)P (specific activity 17.4 Ci/mmol; Amersham, Arlington Heights, IL). After 24-48 h incubation, the medium was removed from the dishes. The cells were then washed with 2 mL of phosphate buffered saline (PBS), which was added to

the culture medium. The washed cells were then lysed in an equal volume of 1% sodium dodecyl sulfate solution. Equal volumes of cells and medium were then extracted with nine volumes of chloroform:methanol (2:1, vol/vol). An aliquot of 0.1 mL of the aqueous phase was dissolved in 10 mL of Aqueous Counting Scintillant (Amersham), and the vials were counted in a liquid scintillation counter (Packard Instruments). The control level of aqueous soluble activity resulting from the incubation of ^{3}H-B(a)P in complete medium without cells was subtracted from the levels obtained with cells.

Results

Induction of SCEs with Direct-Acting Mutagens/Carcinogens

Our initial experiments were designed to demonstrate that the P3 cell line could be used to show induced increases in SCEs after exposure to a few selected direct-acting mutagens/carcinogens. Under our growth conditions, the P3 cell line exhibits a generation time of about 16 h; accordingly, after 36 h of incubation in 5-BudR, the cells have gone through two cell divisions, and harlequin chromosomes are produced.

A previous report (Huberman et al., 1984) described induction of TG-resistant mutants after exposure of the cells to 3-7 μM concentrations of MNNG. In this study, we found a dose-dependent increase in SCEs with 0.6-3 μM concentrations of MNNG (Table I). Higher concentrations of this alkylating agent reduced cloning efficiency by 50% and produced marked delays in the cell cycle.

A similar dose-dependent increase in SCEs could be obtained using another alkylating agent, MNU, at substantially higher concentrations (0.5-2.5 x 10^{-4} M

to 2.5 x 10^{-4} M; see Table I). By contrast, 4-NQO, which is known to produce monoadducts in DNA, yields a dose-dependent increase in SCEs at 2-10 μM concentrations.

The fungal mycotoxin Mitomycin C, known to be a poor mutagen but a potent inducer of SCEs, is likewise effective with the P3 cell line. A dose-dependent induction of SCEs is produced with 3-14 x 10^{-8} M (see Table I of Mitomycin C).

Finally, the active metabolite of B(a)P (7,8-dihydroxy-9,10-tetrahydrobenzo(a)pyrene, BPDE), reported to produce thioguanine mutations in the P3 cells at concentrations of 0.1-0.4 μM, is shown in this study to induce increases in SCEs at concentrations between 8 x 10^{-8} M and 48 x 10^{-8} M (see Table I). Thus, the induction of SCEs by BPDE may occur slightly more frequently than the induction of mutations by this direct-acting carcinogen.

Hydrocarbon-metabolizing activity

It is important to readily identify cells capable of metabolizing procacinogens to the active forms because these cells may be used as activator cells in cocultures with P3 cells. The method of Huberman et al. (1971) provides an easy and rapid assay for measuring the ability of live cells to metabolize PAH to water-soluble products. The assay is proportional to cell number, and production of water-soluble products increases with time.

We have determined that both the BJ-015 (human breast cacinoma) and the RL-12 (rat hepatoma) cell lines are capable of converting ^{3}H-B(a)P to water-soluble products over a 24-h period. Under our conditions of incubation, we estimate that approximately 30-40% of the ^{3}H-B(a)P is converted to the water-

soluble form (data not given). Preincubating the cells with 7×10^{-5} M α-NF inhibits the metabolism by 95% in the RL-12 cells and by about 70% in the BJ cells.

Induction of SCEs in the RL-12 Cells

We have previously demonstrated that the RL-12 cell line can be used to directly measure the induction of SCEs by procarcinogens (Murison et al., 1984). Table II shows that the RL-12 cells will respond to Mitomycin C by exhibiting a high level of SCEs at 3×10^{-8} M. The cells will also respond with induced increases in SCEs when exposed to B(a)P at 0.4×10^{-6} M. Induction of SCEs in the BJ cell line was not attempted because these cells show a much longer cell cycle (generation time estimated to be longer than 24 h) and display an aneuploid karyotype (modal chromosome number 60).

Induction of SCEs in the P3 Cells by Indirect Mutagens/Carcinogens

We have explored some of the conditions under which the target P3 cells respond to indirect carcinogens when cocultured with metabolizing cells. Table III provides a summary of these experiments. Control P3 cells, when cultured alone, either untreated or exposed to B(a)P (0.4 μM) or DMBA (1.4 μM), showed no increase in the level of SCE (see experiments A, B, and C, Table III). P3, cells cocultured with BJ cells but untreated, likewise showed no increase in SCEs (see experiment D). In this experiment, the BJ cells were not killed by irradiation, and their chromosomes were readily distinguishable from those of the P3 cell (see below).

When P3 cells were cocultured with x-irradiated BJ cells (5000 rad) and exposed to B(a)P at 1-2 μM, a dose-dependent increase in SCEs was obtained (see experiments A and B in Table III). Similar cocultures of P3 and BJ cells

exposed to DMBA (1.4 μM) produced an increased level of SCEs. By contrast, exposure of P3 and BJ cells to DMN (6×10^{-4}M) failed to elicit an increase in SCEs (see experiment A). Threefold higher doses were also ineffective (data not shown).

To replace the standard protocol of x-irradiation for inhibition of cell division in the metabolizing cells, we used UV light from a germicidal lamp at a fluence of 40 J/m^2. This treatment was sufficient to inhibit cell division in the metabolizing cells by 90% (see Suzuki et al., 1981). In experiment C, both BJ and RL cells irradiated by UV light and cocultured with P3 cells produced an increase in SCEs after exposure to B(a)P at 2 μM. The cell-mediated activation of B(a)P was allowed to proceed for 24 h.

In nonirradiated BJ cells cocultured with P3 cells, exposure to B(a)P at 1-2 μM again produced dose-dependent increases in SCEs (experiment D). In this case, the P3 chromosomes were readily distinguished from the BJ chromosomes. P3 cells were diploid (2n=46), whereas the BJ cells were hypotetraploid (2n=modal #60). Moreover, the P3 cells had undergone two rounds of cell division, yielding harlequin chromosomes, whereas the BJ cells had divided once and were not harlequinized.

When the BJ or RL cells, previously UV irradiated, were cocultured with P3 cells and exposed to B(a)P at 1-2 μM, a dose-dependent increase in SCEs was observed. The SCE levels in this experiment were somewhat higher than in the previous case with nonirradiated metabolizing cells (experiment D).

In experiment E, when UV-irradiated BJ cells were cocultured with P3 cells and exposed to C(c,d)P and MCA, significant increases in SCEs were observed after exposure to 4.3-12.9 μM C(c,d)P. This response was obtained at

a dose five to ten times higher than the B(a)P dose. Exposure to MCA (0.5 μM) also produced a significant increase in SCEs. By contrast, B(e)P at 40 μM failed to elicit an increase in SCEs.

Discussion

In the present study, we have further characterized the P3 human teratocarcinoma cell line by demonstrating its efficient use in the detection of some direct and indirect mutagens/carcinogens by the induced increase in SCEs.

The P3 cells respond to the direct-acting carcinogens MNNG and BPDE with induced increases in SCEs after exposure to low concentrations of these chemicals. The concentration range is similar to or somewhat lower than that used to produce TG-resistant mutants. Similar studies by Carrano et al. (1978) and Nishi et al. (1984) with 42 different chemicals have clearly shown a close correspondence between the induction of SCEs and mutations. SCEs have also been efficiently induced in the P3 cells by MNU, 4-NQO, and Mitomycin C. The induction of high levels of SCEs in the P3 human teratocarcinoma line with Mitomycin D, however, is contrary to a deficient response produced in a mouse embryonal carcinoma line (Fabricant and Hofnung, 1979).

The P3 cells can also be used to detect indirect carcinogens when they are cocultured with suitable metabolizing cell lines. In this study, both BJ and RL cells were able to convert ^{3}H-B(a)P to the water-soluble form. The RL cell showed increased SCEs after exposure to B(a)P. Coculture of P3 cells with either BJ or RL cells in the presence of either B(a)P or DMBA produced increases in SCEs in the P3 cells. This result was obtained by using live, x-irradiated, or UV-irradiated RL or BJ cells. In the former case, the activator cells (BJ or RL) can be distinguished from the target P3 cells by their respecive karyotypes and by the differences in their respective cell

cycles. The rat cells cycle faster than the P3 cells, and the BJ cells cycle slower. Clearly, this system allows study of the genotoxic responses in both the activator and the target cells by judicious choice of harvesting times. Thus, it may be possible to study tissue and species differences in response to chemical carcinogens.

Finally, we have successfully demonstrated that the P3 cell line can be used to detect the genotoxic effects produced by a number of direct and indirect carcinogens. The induction of SCEs may be correlated with the induction of mutations at the HGPRT locus under incubation conditions similar to those used to achieve metabolic activation of these chemicals.

Acknowledgments

I wish to acknowledge the support and encouragement of Eliezer Huberman and the Chemical Carcinogenesis Group in the Biological, Environmental, and Medical Research Division at Argonne National Laboratory, where the work described in this paper was conducted under a Faculty Research Leave at Argonne appointment. This work supported by the U. S. Department of Energy under contract No. W-31-109-ENG-38.

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Table I. Induction of SCEs in P3 Cells by Direct-Acting Mutagens/Carcinogens

Experiment	Treatment		SCE/Chromosome ± S.E.
A	Control	None	0.207 ± 0.18
	MNNG	0.6×10^{-6}M	0.365 ± 0.10
		3.0×10^{-6}M	0.645 ± 0.12
	4-NQO	2.0×10^{-6}M	0.238 ± 0.06
		10.0×10^{-6}M	0.532 ± 0.10
B	Control	None	0.143 ± 0.05
	MNU	0.5×10^{-4}M	0.319 ± 0.06
		1.5×10^{-4}M	0.436 ± 0.18
		2.5×10^{-4}M	0.604 ± 0.21
C	Control	None	0.173 ± 0.03
	Mitomycin C	3.0×10^{-8}M	0.252 ± 0.08
		2.0×10^{-8}M	0.747 ± 0.12
		14.0×10^{-8}M	0.954 ± 0.23
D	Control	None	0.171 ± 0.04
	BPDE	8.0×10^{-8}M	0.325 ± 0.09
		24.0×10^{-8}M	0.532 ± 0.13
		48.0×10^{-8}M	0.899 ± 0.25

Table II. Induction of SCEs in the RL-12 Cells

Treatment	SCE/chromosome ± S.E.
Control	0.244 ± 0.15
Mitomycin C 3 x 10^{-8}M	1.813 ± 0.73
Benzo(a)pyrene 0.4 x 10^{-6}M	1.860 ± 0.21

Table III. Induction of SCEs by Indirect Mutagens/Carcinogens in P3 cells Cocultured with Metabolizing Cells

Experiment	Cells Cocultured	Treatment		SCE/chromosome ± S.E.	Comments
A	P3 alone	Control	None	0.123 + 0.05	BJ cells irradiated
	P3 + BJ	B(a)P	1.0×10^{-6}M	0.312 + 0.04	5000 Rads x-rays
	P3 + BJ	B(a)P	2.0×10^{-6}M	0.532 + 0.10	
	P3 + BJ	DMN	6.0×10^{-4}M	0.142 x 0.04	
B	P3 alone	B(a)P	0.4×10^{-6}M	0.276 + 0.12	BJ cells irradiated
	P3 alone	DMBA	1.4×10^{-6}M	0.160 + 0.05	5000 rads x-rays
	P3 + BJ	B(a)P	4.0×10^{-6}M	0.521 + 0.05	
	P3 + BJ	DMBA	1.4×10^{-6}M	0.766 + 0.26	
C	P3 alone	Control	None	0.146 + 0.03	BJ and RL cells
	P3 + BJ	B(a)P	2.0×10^{-6}M	0.432 + 0.19	irradiated with
	P3 + RL	B(a)P	2.0×10^{-6}M	0.389 + 0.09	UV 40 Joules/m^2
D	P3 + BJ	Control	None	0.164 + 0.07	BJ cell not
	P3 + BJ	B(a)P	1.0×10^{-6}M	0.423 + 0.10	irradiated
	P3 + BJ	B(a)P	2.0×10^{-6}M	0.620 + 0.21	
	P3 + BJ	B(a)P	1.0×10^{-6}M	0.684 + 0.21	BJ and RL cells
	P3 + BJ	B(a)P	2.0×10^{-6}M	1.033 + 0.28	irradiated with
	P3 + RL	B(a)P	1.0×10^{-6}M	0.562 + 0.29	UV 40 Joules/m^2
	P3 + RL	B(a)P	2.0×10^{-6}M	0.886 + 0.70	
	P3 + RL	DMBA	1.4×10^{-6}M	0.622 + 0.12	
E	P3 alone	Control	None	0.192 + 0.06	BJ cells irradiated
	P3 + BJ	C(c,d)P	4.3×10^{-6}M	0.243 + 0.07	UV 40 Joules/m^2
	$\ell^3 \ddagger \infty >$	$\Psi[\psi' \phi \mu \ell$	$^{12-9} \chi \, ^{10/}$6M	0.303 + 0.08	
	P3 + BJ	MCA	0.5×10^{-6}M	0.381 + 0.12	
	P3 + BJ	B(e)P	40.0×10^{-6}M	0.186 + 0.05	