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CHARACTERIZATION OF CYTOCHROME P450-DEPENDENT AND GLUTATHIONE TRANSFERASE ACTIVITIES IN SV40-IMMORTALIZED UROEPITHELIAL CELL LINES: POSSIBLE ROLE IN TRANSFORMATION AND TUMOR FORMATION

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An in vitro/in vivo transformation system has been developed as a model for bladder tumorigenesis. SV40-immortalized human uroepithelial cells are exposed to putative carcinogens and then implanted into athymic nude mice to test for tumorigenesis. Studies with 4-aminobiphenyl (4-ABP) demonstrated that one cell line, SV-HUC-PC, was sensitive to chemical-induced transformation and another line, SV-HUC-BC, was refractory. We are currently testing this system as a model to identify occupational carcinogens and develop biomarkers of exposure and effects of exposure. As part of this study, we examined P450-dependent metabolism, glutathione transferase, and the effects of chemicals on deoxyribonucleic acid (DNA) synthesis and repair in SV-HUC-PC and SV-HUC-BC. Activities for CYP1A1/1A2, CYP3A, and CYP2B1/2B2 were estimated by determining o*-dealkylation of ethoxy-, benzoxy-, and pentoxy-resorufin, respectively. Coumarin hydroxylase and* p*-nitrophenol hydroxylase were used to estimate CYP2A and CYP2E1, respectively. SV-HUC-PC microsomes had fivefold greater CYP1A1/1A2 activity and twofold higher CYP3A activity than SV-HUC-BC. CYP2B1/2B2 and CYP2A activities and glutathione transferase were not different between the two cell lines. DNA synthesis and repair, by BrdU incorporation, was not different between the two lines when N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) or other reactive metabolites were tested; however, SV-HUC-PC was more sensitive to* n*-nitrosodimethylamine, 4-ABP, and 4,4′-methylene* bis *(2-chloroaniline) (MOCA). The data demonstrate that, while these cells have retained form-specific P450 activities, SV-HUC-PC has greater CYP1A1/1A2 and CYP3A activities.*

Keywords CYP450, glutathione transferase, SV40-transformed uroepithelial cells

Tumors originating in the urothelial tract constitute a significant cancer problem in the United States, with an estimated 52,000 new cases

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annually (Mason et al. 1992). Bladder cancer, the fifth most common cancer in this country, accounted for roughly 11,000 deaths in 1996 (Parker et al. 1996). Although the etiology of bladder cancer is not certain, a number of modifiable host and environmental or occupational risk factors have been identified (Gordon, Helzsouer, and Comstock 1991). An in vitro/in vivo transformation system has been tested with the well-documented human bladder carcinogen, 4-aminobiphenyl (4-ABP; Bookland, Swaminathan, et al. 1992). Previous research identified specific differences in two subpopulations of the SV40-immortalized human uroepithelial cell (HUC) line: SV-HUC-PC and SV-HUC-BC. These cell lines are sensitive and refractory, respectively, to neoplastic transformation and hypoyanthine-guanine phosphoribosyl transferase (HGPRT) mutation by the aromatic amine carcinogen 4-ABP (Bookland, Reznikoff et al. 1992; Bookland, Swaminathan et al. 1992.

The SV-HUC in vitro/in vivo model has been employed by the National Institute for Occupational Safety and Health to assess needed information on putative human chemical carcinogens and to serve as a template on which to develop occupational carcinogen biomarkers. To date, orthophenyl phenol, ortho-toluidine, and 4,4′-methylene *bis* (2-chloroaniline), or their respective reactive metabolites—2-phenyl-1, 4-benzoquinone (PBQ), *N*-hydroxy-*o*-toluidine (N-OH-OT), and *N*-hydroxy-4,4′-methylene *bis* (2-chloroaniline) (N-OH-MOCA)—have been evaluated in this system (Swaminathan et al. 1996). In addition to evaluating the capacity of these chemicals to transform SV-HUC to tumorgenic cells, we also have examined potential biomarkers of occupational bladder carcinogenesis that include deoxyribonucleic acid (DNA) adduct formation, the induction and regulation of SV-HUC ornithine decarboxylase (ODC), and the development of an HUC 2D-PAGE protein database (Debord et al. 1996; Savage et al. 1998; Swaminathan et al. 1996).

The work reported here evaluates differences in metabolism by enzymes important in the biotransformation of potential mutagens or carcinogens. We determined various baseline xenobiotic metabolisms in SV-HUC-PC and SV-HUC-BC and also evaluated differences in cell responses to chemical challenges. Parameters measured included P450 form-specific activities, glutathione transferase, and the DNA synthesis and repair response of each cell line following chemical exposure.

MATERIALS AND METHODS

Cell Culture and Microsome Preparation

Cells were maintained in culture by previously described methods (Bookland, Swaminathan et al. 1992). To obtain cells for microsome preparation, cells were grown to confluence on 100-mm cell culture

plates, then washed with PBS 3 times and 1.0 mL of tissue-preparation buffer was added (25 mM *Tris* [pH 7.5] containing 1 mM dithiothreitol and 0.1*M* EDTA). Cells were then scraped with a rubber policeman, transferred to centrifuge tubes, and disrupted by polytron (30 sec) and sonication (3- to 5-sec bursts). Cell homogenates were centrifuged for 20 min at 14,000 $\times g$. Microsomes were prepared from the homogenates by differential centrifugation as described by Leakey et al. (1989), using a Beckman LS-75 ultracentrifuge. The washed microsomal pellets were suspended in 50 mM *Tris*-HC1 buffer (pH 7.4) without the addition of glycerol. Microsomal protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Xenobiotic Metabolism Assays

P450 form-specific activities of CYP1A1/1A2, CYP3A, and CYP2B1/2B2 were estimated by determining the *o*-dealkylation of alkoxyresorufin substrates (ethoxy-, benzoxy-, and pentoxy-resorufin, respectively) by the method of Burke et al. (1994). Substrate concentrations of 2.0 μM were used for each assay.

Coumarin hydroxylase activity (CYP2A-dependent) was measured as described by Maenpaa et al. (1993). CYP 2E1-dependent, 4-nitrophenol hydroxylase activity was assayed by the method of Reinke and Moyer (1985), as modified by Leakey et al. (1989). Glutathione transferase activities toward 1-chloro-2,4-dinitrobenzene in each cell line were determined by previously described methods (Habig and Jakoby 1981).

DNA Synthesis and Repair in SV-HUC-PC and SV-HUC-BC in Response to Exposure of Cells to Suspected Carcinogens

To determine whether exposure of cells to suspected carcinogens resulted in changes in DNA synthesis, cells were exposed to the test chemicals for 18 h in the presence of BrdU. Cells were plated to 96-well culture plates at 5×10^3 per well in 100 μL. After 4 d, plates were rinsed with PBS and HBBS added along with different concentrations of test chemicals and 10 μM BrdU. After 18 h, media was carefully removed from the cells, wells were rinsed, and BrdU incorporation was estimated by the manufacturer's recommended methods (Boehringer Mannheim Biochemica, Germany).

Statistical Analysis

Significant differences ($p < 0.05$) between in vitro experimental groups were evaluated using ANOVA followed by the least significant difference

TABLE 1. P450-Dependent Metabolism and Glutathione Transferase in SV-HUC-PC and SV-HUC-BC

Cell line	EROD[a]	BROD[a]	PROD[a]	PNP-OH[a]	Coum-OH[a]	GST[b]
SV-HUC-PC	0.57 ± 0.08**	0.29 ± 0.07**	6.03 ± 0.63	2.76 ± 0.10	17.5 ± 1.69	1.7 ± 0.5
SV-HUC-BC	0.17 ± 0.07	0.13 ± 0.03	6.00 ± 0.65	2.56 ± 0.09	15.6 ± 1.81	1.8 ± 0.7

Note. Characteristic activities for CYP1A1/1A2, CYP3A, and CYP2B1/2B2 were estimated by determining *o*-dealkylation of ethoxy-, benzoxy-, and pentoxy-resorufin (EROD, BROD, and PROD), respectively. Coumarin hydroxylase (Coum-OH) and *p*-nitrophenol hydroxylase (PNP-OH) were used to estimate CYP2A and CYP2E1, respectively.

[a] Activities are expressed as pmol product/min/mg protein.

[b] Activities are expressed as units of GST/min/mg protein with 1-chloro-2,4,dinitrobenzene (CDNB) as a substrate. GST, glutathione-*S*-transferase enzyme system.

** $p \leq 0.05$

test. The tests were performed using a SAS General Linear Models program (SAS Institute, Cary, NC) on an IBM personal computer.

RESULTS

Characterization of Cytochrome P450 and Glutathione-Transferase Activities

P450-dependent metabolism and glutathione transferase were determined in SV-HUC-PC and SV-HUC-BC. SV-HUC-PC microsomes had fivefold greater ethoxy-resorufin (CYP1A1/1A2) activity and twofold higher benzoxy-resorufin (CYP3A) activity than SV-HUC-BC. CYP2B1/2B2-, CYP2E1-, and CYP2A-dependent activities and glutathione transferase were not different between the two cell lines (Table 1).

Estimation of DNA Synthesis and Repair

DNA synthesis and repair, by BrdU incorporation, was not different between the two lines when *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) was tested; however, SV-HUC-PC was more sensitive to *n*-nitrosodimethylamine (DMN), 4-ABP, and MOCA (Table 2).

TABLE 2. DNA Synthesis and Repair, by BrdU Incorporation, in SV-HUC-PC and SV-HUC-BC Cells Exposed to *n*-Nitrosodimethylamine (DMN), 4,4′-Methylene *bis* (2-Chloroaniline) (MOCA), 4-Aminobiphenyl (4-ABP), or *N*-Methyl-*N*′-Nitro-*N*-Nitrosoguanidine (MNNG)

Cell line	DMN[a]	MOCA[a]	4-ABP[a]	MNNG[a]
SV-HUC-BC	110	120	120	200
SV-HUC-PC	150	200	200	200

[a] Value represents percent control (vehicle only) absorbance (0.12 A.U.) at the concentration of the respective test substance giving the maximum response. DMN = 100 μM; MOCA = 100 μM; 4-ABP = 200 μM.

DISCUSSION

There are numerous options for in vitro systems to evaluate the carcinogenic potential of chemicals encountered during occupational or environmental exposure. Mutagenesis assays utilizing either prokaryotic (i.e., Ames, *Salmonella* reversion assay) or eukaryotic cells have been well documented and are often the first step in evaluating the genotoxic potential of putative carcinogens (Santella 1987; Sivak 1979). One shortcoming of many mutagenesis assays is that they often are performed with dedifferentiated cell lines that possess few or no characteristics in common with the target organ of the carcinogen to be tested. To overcome this shortcoming, researchers have utilized stable cell lines from various tissues of interest for studying carcinogens of specific organs. Also, because many agents require biotransformation to what is presumed to be the ultimate carcinogen, the capacity for metabolic activities toward the chemicals of interest is often desired. Several studies have suggested that primary HUC and SV40-transformed HUC possess the metabolic capacity to metabolize certain chemicals or their *N*-hydroxymetabolites to DNA-reactive forms (Bookland, Reznikoff et al. 1992; Bookland, Swaminathan et al. 1992; Reznikoff et al. 1986; Swaminathan et al. 1996).

Cytochrome P450 families 1–4 are involved primarily in the metabolism of drugs and other exogenous compounds (Gonzalez 1989). Many compounds that are mutagenic or carcinogenic require activation to a reactive metabolite via cytochrome P450-mediated metabolism (Guengerich 1988). In light of this, when selecting a cell line for use in an in vitro system to screen putative carcinogens, endogenous bioactivation of the test chemical is desirable. Microsomes from SV-HUC-PC and SV-HUC-BC possess measurable P450-dependent activities characteristic of CYP1A1/1A2, CYP3A, CYP2B1/2B2, CYP2A, and CYP2E1. However, SV-HUC-PC had higher levels of CYP1A- and CYP3A-mediated activities than did SV-HUC-BC microsomes. Evidence of the importance of these differences is observed in the increased sensitivity (as measured by increased BrdU incorporation) of SV-HUC-PC to DNA damage from 4-ABP, dimethylnitrosamine, and MOCA. The increased sensitivity of the SV-HUC-PC to two of these three chemicals reflects the increased levels of activity for the P450-forms responsible for bioactivation of 4-ABP (CYP1A1/2) and MOCA (CYP3A; Yun, Shimada, and Guengerich 1992) and, while CYP2E1 is primarily associated with DMN metabolism at low concentrations, both CYP1A and CYP3A may be responsible for bioactivation at higher concentrations. These observations are consistent with earlier reports (Bookland, Reznikoff et al. 1992; Bookland, Swaminathan et al. 1992) detailing differences between HUC-PC and HUC-BC in sensitivity to 4-ABP and a more recent report of neoplastic

transformation of HUC-PC, but not HUC-BC treated with MOCA (Swaminathan et al. 1996).

The glutathione-*S*-transferase enzyme system (GST) facilitates the conjugation of glutathione with nucleophilic agents. This conjugation step usually results in detoxification of the agent and enhances its elimination via bile or urine. In addition to xenobiotic agents, numerous endogenous materials are also conjugated to glutathione by GST. Differences in sensitivity were not likely due to differences in detoxification because glutathione transferase activities in the two lines were not different (Table 1).

In summary, differences in susceptibility to neoplastic transformation and HGPRT-mutation between the SV40-immortalized HUC lines, SV-HUC-PC and SV-HUC-BC, may be related to differences in metabolism toward putative bladder carcinogens. Compared to SV-HUC-BC, SV-HUC-PC was found to possess nearly fivefold more CYP1A-dependent EROD activity and also was significantly more sensitive to genotoxic damage from 4-ABP. Likewise, in the presence of a direct-acting mutagen, MNNG, no difference was seen in the genotoxic response as measured by DNA synthesis and repair in this study and HGPRT-mutation assays by Bookland, Reznikoff et al. (1992); however, the SV-HUC-BC cells did not form tumors even when treated with the penultimate carcinogen, *N*-OH-acetyl aminobiphenyl.

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