BIOMEDICAL AND ENVIRONMENTAL SCIENCES 16, 157-162 (2003)

Anticancer Drug Resistance of HeLa Cells Transfected With Rat Glutathione S-transferase pi Gene[1]

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Objective To establish a cytologic expressing system of rat glutathione S-transferase pi (GST-pi) cDNA for detecting the resistance of HeLa cells to anticancer drugs. **Methods** The assessment was made with various anticancer drugs (adriamycin, mitomycin, cisplatinum and vincristine) that showed different cytotoxicities in transfectant HeLa cells with pSV-GT containing rat GST-pi cDNA (HeLa/pSV-GT) or control pSV-neo (HeLa/pSV-neo). Expression levels of GST-pi mRNA in HeLa/pSV-GT and HeLa/pSV-neo were measured by in situ hybridization using Digoxin-labelled cDNA probe. **Results** HeLa/pSV-GT expressed significantly high degree of GST-pi mRNA, whereas both HeLa/pSV-neo and HeLa cells had very low expression. Cytotoxicities of HeLa/pSV-GT and HeLa/pSV-neo with 4 anticancer drugs were measured by MTT assay. Drug concentrations for yielding 50% inhibition (IC_{50}) in HeLa/pSV-GT by adriamycin, mitomycin and cisplatinum were 70.13 μg/mL, 10.95 μg/mL and 16.52 μg/mL, respectively. In contrast, IC_{50} in HeLa/pSV-neo was 10.34 μg/mL, 7.48 μg/mL and 13.70 μg/mL, respectively. The cytotoxicities of vincristine on both HeLa/pSV-GT and HeLa/pSV-neo were not significantly different. **Conclusions** Our findings suggest that HeLa/pSV-GT containing rat GST-pi cDNA is resistant to some anticancer drugs due to overexpression of GST-pi. Also, HeLa/pSV-GT cell line could serve as a useful cytogenetic model for further research.

Key words: Glutathione S-transferase P1; Enhancer element; Trans-acting factor; Gene transfection; Drug resistance; Tumor cell; In situ hybridization

INTRODUCTION

Glutathione S-transferase (GST) is a superfamily of dimeric isozymes involved in chemical metabolism and material transport in cells and human. There are four major cytosolic types of GSTs, including alpha, mu, pi, theta, and another form, microsomal-GST. Increased expression of GST-pi was detected in precancerous tissues and chemical-induced carcinogenesis, therefore, it appears to be a reliable marker for carcinogenesis. The ability of tumor cells to survive exposure to various anticancer drugs presents the greatest obstacle to successful cancer chemotherapy. The failure of chemotherapy may be due to a variety of

[1] This work was supported by the National Natural Science Foundation of China.

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Biographical note of the first author: Wei CAO, female, born in 1962, majoring in role of Glutathione S-transferase P in tumor drug resistance.

0895-3988/2003
CN 11-2816

causes, and development of drug resistance is possibly one of such causes. The amounts of GST-pi in blood have been shown to correlate with resistance of tumor cells to chemotherapeutic drugs[1,2]. Our previous studies also found that GST-pi was related to anticancer drug resistance. In this study, we transfected HeLa cells with recombinant plasmid containing rat GST-pi cDNA to establish a stable cytologic expressing system for detecting the possible relationship between GST-pi gene expression and resistance of cells to anticancer drugs. The resistance of transfectant HeLa cells to anticancer drugs was assessed by observing the difference in cytotoxicity or sensitivity of transfected cells to cancer chemotherapy.

MATERIALS AND METHODS

Plasmid and Cell Line

pSV-neo and pSV-GT were used for transfection. pSV-GT was made by introducing rat GST-pi cDNA to plasmid pSV in our laboratory. HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum.

Cell Culture and Reagents for Transfection

Cells were cultured in complete medium containing RPMI 1640 (GIBCO), 10% fetal calf serum, HEPES 25 mol/L, glutamine 2 mol/L, penicillin (100 IU/mL) and streptomycin (100 IU/mL). The selection-medium was made by adding G418 Sulfate (500 μg/mL, GIBCO) into complete medium. Suspended cells were obtained with digestion solution containing 0.25% trysin plus 0.02% EDTA.

Radiolabelling of DNA and Detection Kit

The kit was purchased from Boehringer Mannheim Biochemica and radiolabelling was performed as described in the instruction.

Cell Staining Solutions

Routine H-E staining solution.

Reagents for Testing the Resistance of Cells to Drugs

Anticancer agents listed here were obtained from the following companies: adriamycin (ADR, Italian**, lot No. 4017BA); mitomycin C (MMC, Japanese**, value 4%); cisplatinum (CDDP, Chilu Pharmaceutical Factory, China); vincristine (VCR, Guangzhou Mingxing Pharmaceutical Factory, China). 5 mg/mL (3- [4,5-Dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide, Thiazolyl blue), MTT (Sigma) solution and cell lysis solution (20% SDS-50% DMF) were prepared for MTT assay.

Cell Transfection and Selection

HeLa cells were transfected separately with pSV-GT and pSV-neo by the calcium phosphate co-precipitation technique. The transfected HeLa cells were cultured with selection-medium from the 4th day of transinfection. The selected positive clone cells were passagely cultured and stored. The cells transfected with pSV-neo or pSV-GT were named as HeLa/pSV-neo and HeLa/pSV-GT, respectively.

Detection of Expressing Levels of GST-pi mRNA

The GST-pi cDNA was labelled and tested according to the kit instruction. The harvested HeLa, HeLa/pSV-neo and HeLa/pSV-GT were detected by cellular in situ hybridization with 0.5 μg/mL labelled cDNA as described. Hybridized cells were counter-stained with H-E solution and photographed. The expressing levels of GST-pi mRNA were determined by counting the numbers of tiny blue hybridized particles in 100 cells for each cell line. The levels were designated as ± (<10), + (10-30), ++ (30-50) and +++ (>50).

Detection of Resistance of Transfected Cells to Anticaner Drugs by MTT

5×10^4/mL suspended cells were cultured in 96-well plates with 100 cells per well at 37 °C in incubator with 5% CO_2. After the cells were cultured for 24 h, 4 anticancer drugs with various concentrations (as experimental group) and complete medium (as blank control) were added in 3-6 wells, 100 μL per well. The cells were cultured for another 20 h, then added in MTT solution (10 μL per well), and followed by adding cell lysis solution in 4 h later (100 μL per well) to measure the OD_{590} values after staying longer than 4 h.

The resistance of cells to anticancer drugs was determined by cell cytotoxicity and calculated by cell multiplication inhibition tests. The equation was as follow:

Cell multiplication inhibition rate(%) = $[1-(OD_{expt}-OD_{blank})/(OD_{cont}-OD_{blank})]\times100\%$

The relative resistance rate between HeLa/pSV-GT and HeLa/pSV-neo was calculated with equation: RR (%) = IC_{50}(HeLa/pSV-GT) / IC_{50}(HeLa/pSV-neo) × 100%. IC_{50} represented the drug dosage of 50% cell multiplication inhibition rate. All data were expressed as $\bar{x}\pm s$ and analyzed with *t*-test.

RESULTS

Expression of the GST-pi mRNA in Transfected HeLa Cells

A large number of expressed GST-pi cDNA were detected in most HeLa/pSV-GT cells, the levels of expression showed by ±, +, ++ and +++ were 4.12%, 13.40%, 10.28% and 73.20%, respectively; whereas HeLa/pSV-neo and HeLa cells showed little constitutive expression of the GST-pi gene. The levels of ±, +, ++ and +++ were 66.35%, 31.73%, 1.92% and 0.00% in HeLa/pSV-neo, and 59.00%, 41.00%, 0.00%, 0.00% in HeLa cells, respectively.

Cytotoxicity and Aanalysis of Resistance of Transfectant HeLa Cells to Anticancer Drugs

The transfectant HeLa cells' multiplication inhibition rates were proportional to the dose of 4 anticancer drugs (Fig. 1). HeLa/pSV-GT cells were insensitive to adriamycin and mitomycin C when compared with HeLa/pSV-neo, showing decreased cell multiplication inhibition rates. HeLa/pSV-GT cells were also insensitive to cisplatinum with cell multiplication inhibition rate slightly lower than that of HeLa/pSV-neo, but the difference was significant at the point of 4 μg/mL. The cell multiplication inhibition rates in both HeLa/pSV-GT and HeLa/pSV-neo cells were not significantly different.

Statistics showed that the concentrations of adriamycin producing 50% inhibition (IC_{50}) to HeLa/pSV-GT and HeLa/pSV-neo were 70.13 and 10.34 μg/mL, respectively. IC_{50} was elevated by 6.78 times for HeLa/pSV-GT in comparison with HeLa/pSV-neo. Their *RR* was 678.24%, $P<0.05$. Both IC_{50} and *RR* results indicated that the HeLa/pSV-GT cells were

insensitive and resistant to anticancer drug adriamycin. IC_{50} of mitomycin C to HeLa/pSV -GT and HeLa/pSV-neo was 10.95 μg/mL and 7.48 μg/mL, respectively, (*RR* -- 146.39%, $P<0.002$); IC_{50} of cisplatinum to HeLa/pSV-GT and HeLa/pSV-neo was 16.52 μg/mL and 13.70 μg/mL, respectively (*RR* -- 120.58%, and $P<0.005$). From these data, HeLa/pSV-GT cells also showed their resistance to Mitomycin C and cisplatinum, although the resistance was not as strong as to adriamycin. Our results suggested that transfectant HeLa/pSV-GT cells had various levels of resistance to anticancer drugs, such as adriamycin, mitomycin C and cisplatinum.

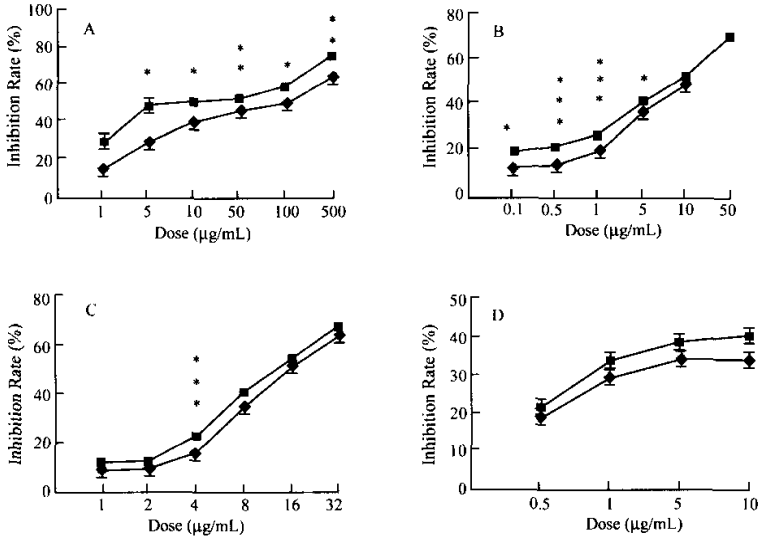

FIG. 1. Inhibitory effect of adriamycin, mitomycin, cisplatinum and vincristine on proliferation of transfected HeLa cells. A: adriamycin (ADR); B: mitomycin (MMC); C: cisplatinum (CDDP); and D: vincristin (VCR). ◆: HeLa/pSV-GT; ■: HeLa/pSV-neo. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$.

DISCUSSION

The intrinsic or acquired resistance of tumor cells to anticancer drugs is a major obstacle to successful cancer chemotherapy. Many different resistant mechanisms have been described so far, which were focused mainly on the changes of ability of drug transport mediated or not mediated by membrane glycoprotein (P-gp), altered topoisomerase II (Topo II) activity and detoxifying activity of metabolizing enzymes (e.g. GSTs, GSH, etc.). All these changes result in decreased intracellular drug accumulation, enhanced drug detoxification and elevated cell ability to survive. GSTs are an important family of dimeric isozymes showing detoxifying activity in cells, and therefore, are involved in anticancer drug resistance. Our result is consistent with current reports[1,3,4], HeLa cells, when transfected with rat GST-pi cDNA and expressed stably, showed resistance to ADR, MMC and CDDP at various levels. The expression of rat GST-pi gene in HeLa cells has induced

the resistance of HeLa/pSV-GT cells to some anticancer drugs. The resistant mechanism is probably due to the fact that the expressed GST-pi increased the detoxification function of drugs.

ADR is a representative anticancer drug of anthracyclines. The resistant mechanism of tumors to ADR is focused largely on increased expression of the multidrug resistance gene (MDR-1), or P-gp mediated cause. But as shown by recent studies, it is unlikely the sole cause of resistance to ADR, since quinone moieties can form conjugation with GSH through GST catalysis, and moreover, GST can remove free radicals. Quinone-mediated free radicals formed from ADR can be detoxified by GST-pi. This detoxification by GST-pi can act as a cellular protective effect, thus, increasing cellular resistance to ADR[3]. Some experimental cell lines, like our HeLa/pSV-GT cell line, demonstrate that the increased expression of GSTs, especially GST-pi, is related to the resistance of cells to ADR[1,4,5]. In contrast, it was reported that the expression of GST-pi mRNA in some transfectant cell lines did not produce significantly enhanced drug resistance[6]. It is understandable that P-gp and GST-pi are two separate systems responsible for cellular resistance to ADR, which function conditionally, and are not necessarily essential to anticancer drug resistance.

MMC is an alkylating agent of anticancer property. Its cytotoxicity becomes complete through alkylation and formation of quinone-mediated free radicals. Phase II (GST) can catalyze a more water-soluble conjugate with these hydrophilous radicals in drug metabolism, which may be less toxic and more readily excretable. CDDP is known as an agent not to be the substrate of P-gp, because CDDP penetrates, instead of being transported into cells[7]. The cellular detoxification function of CDDP is unclear, although some experiments have revealed that GST-pi is related to resistance of cells to CDDP[5,7]. Our results have also shown that the expression of GST-pi enhanced the resistance of transfectant HeLa/pSV-GT cells to MMC and CDDP (by 1.5 and 1.3 times, respectively.). We could not detect any resistance to VCR, an anticancer drug belonging to vinca alkaloids, which is consistent with other reports[5]. The resistant mechanism of VCR is mainly considered to correlate with the altered functions of cellular VCR-transporting proteins.

From the results of recent researches on resistance of tumors, it is concluded that neither P-gp nor GST-pi can serve as a complete interpretation of resistant mechanisms of tumors to anticancer drugs. For example, the increased expression of GST-pi can conditionally enhance resistance at different levels for some tumors to various anticancer drugs, also, the structures and properties of various anticancer drugs induce different cellular responses. Therefore, the development of drug resistance mediated by GST-pi is complicated, and a variety of mechanisms are involved.

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(*Received October* 25, 2002 *Accepted January* 20, 2003)