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GST-π EXPRESSION IN TRANSFORMED CELLS BY TRANSFECTING OF DNA ISOLATED FROM HUMAN FETAL LUNG TISSUES TREATED WITH CARCINOGENS*

Yao Denggao 姚登高 Hu Guogang 胡国刚 Luo Xianmao 罗贤懋
Zhu Ming 朱明

Cancer Institute, Chinese Academy of Medical Sciences, and Peking Union Medical College, Beijing 100021

Objective : To investigate the relationship between the GSTs, GST-π expression and initiation of lung carcinogenesis. Methods: The Rat-1 cells were transformed by carcinogens (DEN, MNU and CSC) treated fetal lung DNA for 24 h. Results: The GSTs activities toward 1-chloro-2, 4-dinitro-benzene (CDNB) in transformed cells were significantly higher than in the solvent control cells ($P<0.05$). GST-π content and GST-π mRNA expression level of transformed cells were also higher than those of control cells which were performed by ELISA and Northern blotting method respectively. The results indicated that the higher GSTS activities of transformed cells were due to the increase of GST-π content and the GST-π mRNA overexpressing may be responsible for the increase of GST-π protein level of the transformed cells. Conclusion: The changes of GSTs and GST-π may be considered as the one of the biomarkers of the initiation of human lung carcinogenesis.

Key words: GSTs, GST-π, Lung carcinogenesis, Initiation marker.

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Abbreviations: GST, glutathione S-transferase; GST-π, GST pi class; DEN, diethylnitrosamine; MNU, N-methyl-nitrosourea; CSC, cigarette smoke condensate; HFL, human fetal lung; CDNB, 1-chloro-2,4-dinitro-benzene.

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a group of multifunctional dimeric cytosolic enzymes which were originally identified as enzymes involved in drug metabolism. Three isoforms in human tissues were termed α, μ and π according to their structures, enzymatic kinetics and immunological specificities.[1] Liver tissue contains large amounts (up to 4% of the cytosolic protein) of GST α, and kidney, testis and adrenal also express α as the major form.[2] It has been reported that individuals lacking GST-μ are more likely to contract lung cancer which indicated that it is an enzymatic marker for susceptibility to lung cancer among smokers.[3] As one major form of GSTs, GST-π expression level is less individual than other isoenzymes which suggested that this enzyme may play an important role in the aetiology of malignant disease.[4] Overexpression of the π class has been associated with tumor development and carcinogenesis.[5]

GST-P can be used as a marker for chemically induced hepatocarcinogenesis in rat, this form of GST is minimally expressed in normal rat liver but is induced several fold in chemically induced preneoplastic and neoplastic lesions.[6] GST-π of human has structural and immunological similarities with the GST-P of rat. The relationships between GST-π and smoking have been investigated by many groups.[3,7] The higher expression of GST-π was detected in the transformed cells by carcinogens-treated human fetal

lung DNA than in untransformed cells which performed by immuno-Ag-Au assay in our laboratory, but the correlation between GSTs activities, GST-π protein level and the molecular mechanism of GST-π expression in transformed cells needs further investigation. Although some studies have suggested the possibility of GST-π being used as a marker for human tumor,[8] whether it can be used as a biomarker for chemically induced lung tumors also needs to be confirmed. In the present study, GSTs activity, GST-π content and GST-π mRNA expression level in the transformed cells by carcinogens-treated human fetal lung DNA were compared with untransformed cells.

MATERIALS AND METHODS

Materials

Rat anti-rabbit monoclonal antibody against GST-π (MAb III) and rat anti-human polyclonal antibody against GSTs were produced in our laboratory. pUC18 plasmid containing GST-π cDNA fragment was a generous gift by professor Muramatsu, University of Tokyo, Japan. Random priming kit was purchased from Promega, N-methylnitrosourea (MNU) and diethylnitrosamine (DEN) were purchased from Fluka, cigarette smoke condensate (CSC) was provided by Dr Gu Mei, Rat-1 cell line was a gift by Professor Li Shengde, Cancer Institute, CAMS/PUMC.

Human Fetal Tissue Treated with Carcinogens

Human fetal lung (HFL) tissues were obtained within 6 h after abortion followed by washing with ice-cold L-15 medium and cut in 1.5×1.5×1.0 mm small pieces using scalpel and were cultured in M199 medium for 24 h, then treated with carcinogens CSC (100 μg/ml), DEN (7 μl/ml), MNU (100 μg/ml) and solvent DMSO (5 μl/ml) for 24 h respectively. The treated tissues were washed with PBS and then stored in the liquid nitrogen for DNA isolation.

Transfection, Soft-agar Culture and Single Colonies Screening

Genomic DNA was isolated from treated tissues and transfected into Rat-1 cells using DNA-Ca_3 $(PO_4)_2$ coparticipate method. Transformed foci were screened out by growing the cells in low-FBS (2.5%) medium and transformed cells were further cultured and selected in soft-agar. Final clones were picked out from soft-agar and continuously cultured for following experiment.

GSTs Activity Assay and GST-π Content Determining by ELISA

GSTs activity towards 1-chloro-2, 4-dinitrobenzene (CDNB) was analysed as described by Habig.[9] The multiwell plates were covered overnight at 4℃ in covering buffer containing diluted (1:700) primary antibody (MAb III) following blocked at 37℃for 30 min. Samples or 100 μl of standard antigens were added in each well to bind with GST-π antibody at 37 ℃ for 2 h, then GSTs antibody was added and incubated at 37℃for 2 h. HRP anti-rabbit IgG from donkey was then added and incubated at 37℃ for 90 min. The color was produced by adding 4-nitrophenol phosphate and standing at room temperature for 1 h and reaction was stopped by adding the same volume of 3 mol/L NaOH. Absorbance was obtained at a wavelength of 410 nm on the spectrophotometer.

RNA Isolation and Northern Blotting

Total cellular RNA was isolated according to the method of Sambrook et al,[10] 30 μg of total RNA was electrophoresed through a 1.2% agarose-formaldehyde gel, transferred to nitro-cellulose membrane and then was hybridized with ^{32}P-dCTP labeled GST-π cDNA (0.75 kb) by random-priming method. After washing, the membrane was autoradioexposed at -70 ℃ and developed.

RESULTS

Transformed RAT-1 Cells by Human Fetal Lung (HFL) DNA Treated with Carcinogens

Comparing transformed cells by HFL DNA treated with carcinogens with normal Rat-1 cells, transformed cells have the properties of cancer cell such as rapid and uncontrolled rate of growth, big and deep color nuclei and lacking the density-dependent inhibition to divide to ever-increasing densities (Figure 1). They even could invade into the surrounding cells. Untreated and DMSO treated cells were not transformed and didn't have these properties (Figure

2). The transformed cells were further confirmed by growing them in soft-agar.

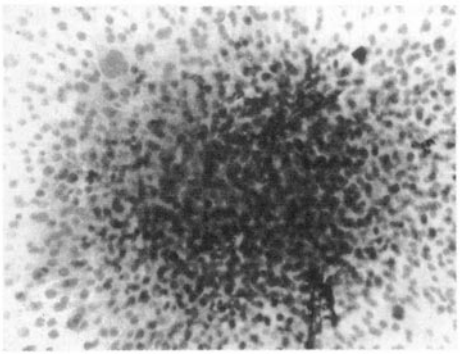

Fig. 1. Transformed Rat-1 cells transfected with HFL exposed to CSC. The cells containing big and deep nuclei, and lacking the density-dependent inhibition with irregulative growth.

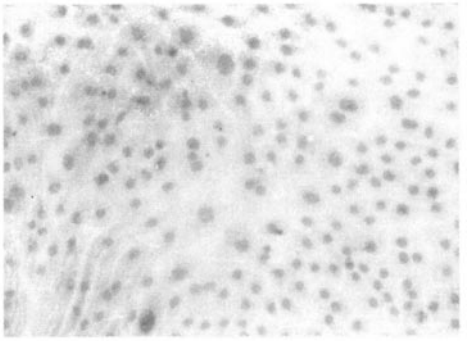

Fig. 2. Control Rat-1 cells show polyhedral with single-layer growth.

GSTs Activity Towards CDNB and GST-π Content

Significant differences were observed between all three transformed cell lines and solvent treated cell line in both of GSTs activities towards CDNB (Table 1) and GST-π content (Table 2). Among the carcinogens treated cell lines, the orders of GSTs activities and GST-π content are DEN>CSC>MNU.

The Expression of GST-π mRNA

Using Northern blotting hybridization of total cellular RNA from different cell lines with ^{32}P-labelled GST-π cDNA probe, mRNA expression was carried out in all carcinogens treated cell lines, but wasn't detected in solvent of DMSO treated cell line and the level of GST-π mRNA expressing is correlated with GSTs activities and GST-π content which were shown above (Figure 3).

Table 1. GSTs activity towards CDNB in transformed cells by human fetal lung DNA treated with carcinogens

Treatment	No. of experiments	GSTs activity (nmol/min/ml) $\bar{x}\pm s$	*P*
DMSO	9	33.0± 12.9	
CSC	8	66.4± 23.5	<0.05
DEN	6	86.6± 31.0	<0.05
MNU	7	54.2± 13.6	<0.05

Table 2. GST-π content in transformed cells by human fetal lung DNA treated with carcinogens

Treatment	No. of experiments	GST-π content (ng/ml) $\bar{x}\pm s$	*P*
DMSO	6	336.4± 37.4	
CSC	6	1163.6± 421.3	<0.05
DEN	6	1628.7± 104.3	<0.05
MNU	6	640.7± 186.2	<0.05

DISCUSSION

Carcinogenesis is a multistage process including initiation, promotion and progression driven by genetic damage and epigenetic changes.[11] The initiation is the unreversible alterations of large molecules such as DNA which are induced by carcinogens and the initiated cells are potential cancer cells. Lung cancer is the major type of cancer in the world, the mortality rate of lung cancer has been increasing steadily since 1930.[12] Therefore, the good model of human lung cancer initiation is very useful for study of human lung carcinogenesis, and then evaluating the chemoprevention strategy, early diagnosis and clinic trail.

GSTs and GST-π expression have been detected in many kinds of neoplastic and preneoplastic lesions.[13,14] Most of these works have been done in animal model initiated by carcinogens. Transfecting

DNA treated with carcinogens into Rat-1 cells, transformed cells were screened out and GST-π higher expression in transformed cells than in the control cell was detected by Immuno-Au-Ag in our laboratory. Using this model, higher GSTs activities, higher content of GST-π and higher expression level of GST-π mRNA also were detected in transformed cells than in control by activity assay, ELISA and Northern blotting respectively.

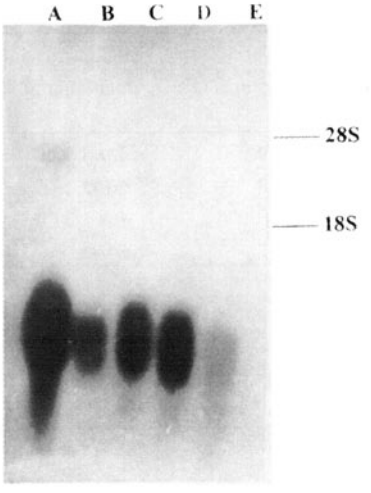

Fig. 3. Detection of GST-π mRNA of cells by Northern blot

A. MCF-7 ADR cells (GST-π mRNA expression high)
B. Transformed Rat-1 cells transfected with HFL exposed to MNU
C. Transformed Rat-1 cells transfected with HFL exposed to CSC
D. Transformed Rat-1 cells transfected with HFL exposed to DEN
E. Transformed Rat-1 cells transfected with HFL exposed to DMSO as control

GSTs are a group of phase II conjugating enzymes which play a central role in the inactivation of toxic and carcinogenic electrophiles. GST-π is the most ubiquitous of the human GST family, being expressed in all tissues examined.[4] The initiation of hepatocytes by genotoxic carcinogens gave rise to foci of altered gene expression, such as GST-π.[15] GST-π content detected in transformed cells was compared with control cells in the present study, as we expected that all transformed cell lines have higher GST-π content than untransformed cell line which indicated that GSTs activities increase may be caused by the GST-π protein level. Further investigation done by Northern blotting showed that mRNA expressing level of GST-π was higher in transformed cell lines than in the control cell line and the order of expression level was correlated with the content of GST-π in all three transformed cell lines, i.e., DEN>CSC>MNU.

Our present results indicated that GSTs activities have been changed during the initiation of human lung carcinogenesis. GST-π as one isoform in the human which has structural similarity with GST-P in the rat was found to be a major form which caused the increasing of GSTs activities. The fact that GST-π mRNA expression level in the transformed cells was higher than in the control cells suggested that high GST-π protein content in the transformed cellswas the result of gene control in transcription level. GST-π is hopeful to be a biomarker of initiation of human lung carcinogenesis as the GST-P of rat.

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