Original Article

Experimental Study on Malignant Transformation of Human Bronchial Epithelial Cells Induced by Glycidyl Methacrylate and Analysis on its Methylation*

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Abstract

Objective To establish the model of human bronchial epithelial cells (16HBE) malignant transformation induced by glycidyl methacrylate (GMA) and define the different methylation genes at different stages.

Methods DNA was extracted at different 16HBE malignant phases and changes of genes DNA methylation at different stages were detected using Methylation chip of 'NimbleGen HG18 CpG Promoter Microarray Methylation'. Methylation-specific PCR (MSP) was used to observe the methylation status of some genes, and then compared with the control groups.

Results The result showed that GMA induced 16HBE morphorlogical transformation at the dose of 8 μg/mL, and cell exposed to GMA had 1 374 genes in protophase, 825 genes in metaphase, 1 149 genes in anaphase, respectively; 30 genes are all methylation in the 3 stages; 318 genes in protophase but not in metaphase and anaphase; 272 genes in metaphase but not in protophase and anaphase; 683 genes in anaphase but not in metaphase and protophase; 73 genes in protophase and metaphase but not in anaphase; 67 genes in protophase and anaphase but not in metaphase; 59 genes in metaphase and anaphase but not in protophase.

Conclusion The pattern of DNA methylation could change in the process of 16HBE induced by GMA.

Key words: DNA methylation; Chip; Glycidyl methacrylate; 16HBE

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INTRODUCTION

Human may be in danger of exposing to low dose of chemical. Glycidyl methacrylate (GMA) is an important chemical widely used in resin, coating, adhesive, and plastic industries. The purpose of this study was to investigate the methylation in human bronchial epithelial cells malignant transformation induced by glycidyl methacrylate.

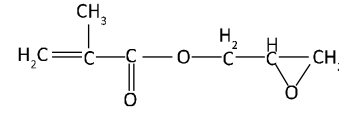

Figure 1. The chemical structure of GMA.

In the late 1980s, we firstly reported that GMA had mutagenicity in China, and found that GMA showed evident genotoxic and epigenotoxic effects in various systems *in vivo* and *in vitro*, including

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primary DNA damage, chromosomal damage, and gene mutations. We also found that GMA could induce malignant transformation in several types of mammalian or human cells.

Results from our previous research showed that GMA is a new mutagen and can induce neoplastic transformation of BALB/c 3T3 cells *in vitro*, thus, we suggested the potential carcinogenicity of this compound. In the previous researches of 16HBE exposed to GMA, we found that GMA could induce cell mutation and malignant transformation, and the early genetic effects of GMA were mainly on bacteria reverse mutation, DNA damage, cell cycle and apoptosis in cultured human bronchial epithelial cells (16HBE). The malignant transformation model of 16HBE induced by GMA was also successfully established *in vitro*. This research system provides a potential tool for the study of cell and molecular mechanism in the multistage carcinogenesis of this chemical. In the researches of 16HBE induced by GMA, we also found that GMA affected the function of the genes by damaging the cell chromosome. The defect of DNA repair system induced by GMA and the imbalance of injure- repair led to the cell transformation. And on the other hand, the changes of genes and pathways were also studied.

Up to date, the molecular mechanism of 16HBE malignant transformation induced by GMA is still unclear, so the purpose of this study was to find the pattern of DNA methylation changed in the process of 16HBE malignant transformation induced by GMA. Different from the classical genetic theory, DNA methylation plays an important role in the process of cells malignant transformation, gene regulation, cell proliferation, gene function and so on[1-4]. It was also considered to be the new tumor molecular biological markers (biomarker). In order to provide new evidences of carcinogenic characteristics of GMA, an epidemiologic monitoring and mechanism study of its chemical carcinogenesis was conducted in the present study.

MATERIALS AND METHODS

Chemicals

GMA (The purity ≥98.5%, Dow) was obtained from GUANG ZHOU SHUANGJIAN TRADING CO., LTD., the others were culture medium (Hyclone), trypsin (Amresco), Quick-gDNA™ MiniPre (Zymo, America), $EDTANa_2$ and DMSO (Sigma), NaCl, KCl, NaOH and so on (Beijing, China).

Cell

Human bronchial epithelial cells (16HBE) were obtained from California University.

Animal

The nude mice, Balb/c, weight 16-18 g, were purchased from Vital River Laboratory Animal Technology Co., Ltd.

Culture and Transformation

The 16HBE were cultured with minimum essential medium supplemented with 4 mmol/L glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum. The cells were cultured exponentially, plated at the density of 5.0×10^5 and exposed to GMA at the dose of 8 μg/mL for 72 h. Same volume of DMSO was used as solvent. Then the cells were washed with PBS, detached by trypsinization and then plated to another culture bottle for another two GMA exposures at same dose and for same time period respectively. Subsequently, the GMA induced cells were signed for the first generation and cultured with medium of 5% FBS till the 30^{th} generation. All the cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. They were subcultured till cells grown more than 90% using 0.25% trysin.

The cells agglutination activity with low concentration of conA and agglutination time induced by GMA were observed. Because the transformed cells with anchorage independence could grow in semi-solid agar and showed a dose-reaction relationship with the concentration of GMA, we collected cells at the 10^{th}, 20^{th}, and 30^{th} generation. Then the malignant transformation model of 16HBE induced by GMA *in vitro* was established. During the culture, the cells were identified by the tests of conA, colony forming frequency on soft agar, scanning electron microscope and tumorigenesis in nude mice. Test of immunocytochemical detection was also applied to confirm the derivation of cell and tumor formation.

Methylation Chip

The changes of DNA methylation at different stages were detected using the methylation chip of 'NimbleGen HG18 CpG Promoter Microarray Methylation'.