Original Article

Effect of Mono-2-ethyhexyl Phthalate on DNA Methylation in Human Prostate Cancer LNCaP Cells*

WU Jian Hui[1,2,3,&], CHEN Jiao[4,5,&], WANG Yong[1,2,3], XIA Bin[5], WANG Rong[1,2,3], ZHAO Yan[5], WANG Qin Xia[1,2,3], SONG Qi[5], YAO Shun Heng[1,2,3], ZHANG Yun Hui[5,#], and SUN Zu Yue[1,2,3,#]

1. National Evaluation Centre for the Toxicology of Fertility Regulating Drug, Department of Pharmacology and Toxicology, Shanghai Institute of Planned Parenthood Research, Shanghai 200032, China; 2. Key Laboratory of Reproduction Regulation of NPFPC, Shanghai 200032, China; 3. Reproductive and Developmental Research Institute of Fudan University, Shanghai, Shanghai 200032, China; 4. Division of Health Risk Factors Surveillance and Control, Shanghai Municipal Center for Disease Control and Prevention, Shanghai 200336, China; 5. Key Laboratory of Public Health Safety, Ministry of Education, School of Public Health, Fudan University, Shanghai 200032, China

Abstract

Objective To evaluate whether mono (2-ethylhexyl) phthalate (MEHP) affects genomic DNA methylation and the methylation status of some specific genes such as patched gene (*PTCH*) and smoothened gene (*SMO*) in LNCaP cells.

Methods LNCaP cells were treated with MEHP (0, 1, 5, 10, and 25 μmol/L) for 3 days. An ELISA assay was preformed to detect genomic methylation, including 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) content. A pyrosequencing assay was applied to assess DNA methylation in *PTCH* and *SMO* gene promoters. The correlation between DNA methylation and gene expression was assessed.

Results The proportion of cytosines with 5-mC methylation in LNCaP cells was significantly decreased by MEHP (1, 5, 10, and 25 μmol/L) in a dose-dependent manner ($P < 0.01$). For genes in the Hedgehog pathway, there was no significant MEHP concentration-dependent difference in the DNA methylation of *PTCH* and *SMO*.

Conclusion MEHP might affect the progression of prostate cancer through its effect on global DNA methylation.

Key words: Mono (2-ethyhexyl) phthalate; LNCaP; Genomic; Methylation; *PTCH*; *SMO*

Biomed Environ Sci, 2017; 30(9): 641-648 *doi: 10.3967/bes2017.085* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

*This study was supported by the National Science and Technology Major Projects for 'Major New Drugs Innovation and Development' (No. 2011ZX09301-003); and partly supported by The Fourth Round of Three-year Public Health Action Plan of Shanghai (No. 15GWZK0202).

&These authors contributed equally to this work and should be considered co-first authors.

#Correspondence should be addressed to ZHANG Yun Hui, E-mail: yhzhang@shmu.edu.cn; SUN Zu Yue, E-mail: sunzy64@163.com

Biographical notes of the first authors: WU Jian Hui, born in 1976, male, doctoral degree in pharmacology, majoring in reproductive pharmacology & toxicology; CHEN Jiao, born in 1990, female, master degree in medicine, majoring in environmental pollution.

INTRODUCTION

Prostate cancer (PCa) is the most common malignant tumor in men, being the second leading cause of deaths from cancer in men[1]. According to cancer statistics from the National Institutes of Health (NIH), PCa is most frequently diagnosed among men aged 65-74 years[2]. PCa patients are initially treated with androgen ablation therapy that causes regression of androgen-dependent tumors. However, prolonged androgen deprivation generally results in relapse of androgen-independent tumors and is inevitably fatal[3].

Patients undergoing various medical procedures are inevitably exposed to di (2-ethylhexyl) phthalate [also known as Bis (2-ethylhexyl) benzene-1, 2-dicarboxylate, DEHP], a major plasticizer compound that is added to polyvinyl chloride (PVC)-based medical materials. The Food and Drug Administration (FDA) has assessed the safety of DEHP released from PVC-based medical devices and pointed out the necessity to consider the potential adverse effects of DEHP in exposed patients. It has been pointed out that an adult undergoing extracorporeal membrane oxygenation (ECMO) could receive a DEHP dose ≥ 4 $mg \cdot kg^{-1} \cdot d^{-1}$, which is much higher than the tolerable intake of external exposure (0.6 $mg \cdot kg^{-1} \cdot d^{-1}$)[4]. Further, mono (2-ethylhexyl) phthalate (MEHP, the primary toxic metabolite of DEHP) can affect steroidogenesis, decrease the activity of androgen, and induce apoptosis in Leydig cells; it can also induce DNA oxidative damage through the activation of ROS production[5-8]. Moreover, MEHP has been shown to induce matrix metalloproteinase 2 (MMP2) expression that was strongly associated with tumor metastasis and progression in testicular embryonal carcinoma NT2/D1 cells[9]. Exposure of male rats to DEHP has been shown to induce the increase in prostatic volumes and weight, as well as the prostate index with expanded glands, and the degree of prostatic hyperplasia showed a positive correlation with the DEHP dose[10]. Our previous findings showed that MEHP (1-25 μmol/L) activated the expression of critical genes (patched homolog 1, *PTCH1*; and smoothened homolog, *SMO*) in the Hedgehog signaling pathway in androgen-sensitive human prostate adenocarcinoma LNCaP cells derived from a metastatic lesion of a human prostatic carcinoma, indicating that MEHP might advance the progression of PCa[11].

It is commonly believed that DNA methylation is the major epigenetic regulatory mechanism of gene expression and is involved in the progression of PCa[12]. For the present study, genome-wide DNA methylation and methylation of specific genes was considered. A reduction in genomic methylation intermediates, such as 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), can result in tumor heterogeneity and relapse[13-14]. Moreover, the abnormal methylation status of specific genes can induce gene overexpression or silencing, accelerating the proliferation and metastasis of cancer. There is a great deal of evidence supporting that DNA methylation of *PTCH* and *SMO* in the Hedgehog pathway is involved in the progression of cancers[15-17].

Therefore, this study was designed to investigate the effect of MEHP on DNA methylation through ELISA and pyrosequencing assays, including an assessment of the relationship between 5-mC and 5-hmC content in genomic DNA and the methylation status of specific genes, *PTCH* and *SMO*. Further, the aims of the study included exploring whether MEHP can affect DNA methylation in LNCaP cells, as well as the potential role of DNA methylation in the progression of PCa.

MATERIALS AND METHODS

Cells and Reagents

The human PCa cell line LNCaP (Cat. No. TCHu173) was obtained from the Chinese Academy of Science Cell Bank (Shanghai, China). Fetal bovine serum (FBS), charcoal-stripped FBS (CS-FBS), RPMI1640 (phenol-red free), and 0.25% Trypsin-EDTA were bought from Gibco (Grand Island, NY). MEHP and dimethylsulfoxide (DMSO) were obtained from Sigma (St. Louis, MO) and TRC (Toronto, Canada) respectively. Gene primers were synthesized by Sangon Biotech (Shanghai, China). The QIAamp® DNA Mini Kitwas from Qiagen (Hilden, Germany). The 5-mC DNA ELISA Kit, Quest 5-hmC™ DNA ELISA Kit, and EZ DNA Methylation™ Kit were from Zymo Research (Orange, California). GoTaq® Hot Start was obtained from Promega (Madison, USA). PyroMark® Gold Q96 Reagents were bought from Qiagen (Germany).

LNCaP Cell Culture and Treatment with MEHP

LNCaP cells were cultured in RPMI1640 containing 10% FBS in a humidified atmosphere of