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Urinary 1-hydroxypyrene and smoking are determinants of LINE-1 and AhRR promoter methylation in coke oven workers

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ABSTRACT

Coke oven emissions (COE) containing polycyclic aromatic hydrocarbons (PAHs) are predominant toxic constituents of particulate air pollution that have been linked to increased risk of lung cancer. Aberrant DNA methylation is one of the best known epigenetic changes in human cancers and healthy subjects exposed to carcinogens. The purpose of this study is to explore the factors influencing the methylation of long interspersed nuclear element-1 (LINE-1) and aryl-hydrocarbon receptor repressor (AhRR) in coke oven workers. The study population is composed by coke oven workers (348) and water treatment workers (131). And their urinary PAH metabolites were analyzed by high performance liquid chromatography; DNA methylation were measured by pyrosequencing. The urinary PAHs metabolites were significantly elevated in coke oven workers ($P < 0.01$). The results from multivariate logistic regression analysis showed that a high level of urinary 1-hydroxypyrene was associated with a significantly increased risk of hypomethylation of LINE-1 (OR: 1.80; 95% CI: 1.25, 2.60), and heavy smoking was associated with a significantly increased risk of hypomethylation of AhRR (OR: 1.44; 95% CI: 1.04, 2.00). Our findings demonstrate that urinary 1-hydroxypyrene may be a useful biomarker for evaluating the role of PAHs exposure on hypomethylation of LINE-1 among coke oven workers and that smoking may be an important factor affecting hypomethylation of AhRR.

1. Introduction

Coke oven workers are exposed to coke oven emissions (COE) that contain a wide variety of volatile organic solvents and particulates, especially polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous environmental and occupational pollutants, and a recent reassessment of their carcinogenic potential led to BaP being upgraded to a Group 1 known human carcinogen by the International Agency for Research on Cancer (IARC) [1]. Epidemiological studies suggest an aetiological link between carcinogenic PAHs exposure and lung cancer risk in coke oven workers exposed to COE, and coke oven workers were found to have three to seven-folds increased risk for developing lung cancer [2–4].

As a complex disease, cancer arises from both genetic and epigenetic errors. While the importance of genetic alterations in cancer, including chromosomal instability and genetic mutations, is evident, aberrant epigenetic regulation in malignant cell transformation is still poorly understood. Aberrant DNA methylation is one of the best known epigenetic changes in human cancers [5–7]. Aberrant promoter methylation of a series of tumor suppressor genes has been detected in blood leukocyte DNA from cancer patients and healthy subjects exposed to carcinogens [8,9].

Previous studies indicate that aryl-hydrocarbon receptor repressor (AhRR), a basic helix-loop-helix/Per-ARNT-Sim (bHLH/PAS) family transcription factor, inhibits aryl-hydrocarbon receptor (AhR) function by competing with AhR for dimerizing with AhR nuclear translocator (ARNT) and binding to the xenobiotics responsive element (XRE) sequence. Thus, AhR and AhRR form a kind of regulatory biofeedback circuit in the xenobiotic signal transduction pathway [10,11]. Activation of procarcinogenic PAHs to ultimate carcinogens by AhR regulated enzymes is traditionally considered the first step in tumor initiation [12]. However, a DNA methylation alteration in AhRR gene induced by ambient PAHs, which may be involved in air pollution related to lung carcinogenesis, has not been examined.

Global DNA hypomethylation, particularly in repeated sequences and transposable elements, largely affects intergenic and intronic regions of the genome and plays a critical role in increasing chromosomal instability [13,14]. Long interspersed nuclear element 1 (LINE-1) represents a family of non-long terminal repeat retroposons that are interspersed all over the genomic DNA and account for approximately 20% of the human genome. The level of LINE-1 methylation is regarded as a surrogate of global DNA methylation for its high frequency in the

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genome [15,16]. Quantitative methylation analysis on LINE-1 in lymphocytes is a useful marker for evaluating the effect of environmental and occupational pollutants on the genome [17].

In the present study, we investigated the effects of chronic high-dose PAHs exposure on global and specific gene methylation modification by quantitating LINE-1 and AhRR levels in peripheral lymphocyte DNA of subjects with well-characterized PAHs exposure.

2. Materials and methods

2.1. Study subjects

A total of 479 subjects were recruited from a steel plant in northern China, of whom 348 workers have worked on top-, side- and bottom-oven in the coke oven plant, which led to regular exposure to COE, and these workers had been employed for at least one year. The other 131 subjects from the department of water treatment with no related PAHs exposure in their workplace were used as control subjects. The subjects were not exposed to known mutagenic agents, such as radiotherapy and chemotherapy in the last three months. A pre-tested questionnaire on demographic characteristics, smoking history, alcohol consumption, history of occupational exposure and family medical history were administered in person by trained interviewers.

Smoking was categorized as current, former, and non-smoker. Current smokers were defined as those who smoked at least one cigarette per day in the last six months. Former smokers were defined as those who used to be smokers, but who had smoked less than one cigarette per day or stopped smoking for at least the past six months. The rest of the participants were defined as non-smokers. Drinking was categorized as current drinkers who drank hard liquor, beer, or wine at least one time per week during the past six months. Former drinkers were those who used to be drinkers, but who had drank less than one time per week or stopped drinking for at least the past six months. Non-drinkers are those who always drank less than once per week or not at all. This study was approved by the Medical Ethics Committee of the Shanxi Medical University. All samples were analyzed without knowing the subject status.

2.2. Urinary creatinine and hydroxyl-PAH measurements

According to the recommendation of the American Conference of Governmental Industrial Hygienists (ACGIH), the morning urine samples of end-of-work-week were obtained. All urine samples were stored at −80 °C until analysis. The detailed analytical method is described elsewhere [18–21]. The sample was analyzed by using high performance liquid chromatography (HPLC, Shimadzu Corp, JPN) equipped with a fluorescence detector to determine 2-hydroxynaphthalene, 2-hydroxyfluorene, 9-hydroxyphenanthrene and 1-hydroxypyrene levels. The linearity (expressing as R^2), limit of detection (LOD), reproducibility (expressing as coefficient of variation (CV)) and mean recovery rate were 0.9998–1, 0.04–0.12 μg/L, 2.04%–4.27%, and 82.97%–107.85% respectively. Urinary creatinine was detected with alkaline picrate, and the creatinine-picrate complex was quantified by spectrophotometry (SpectraMAx M2, Molecular Divices, USA) using a wavelength of 520 nm. The concentrations of these four hydroxyl-PAHs were presented in units of μg/mmol creatinine. The ΣOH-PAHs represent the sum of four PAHs metabolites we measured.

2.3. Bisulfite treatment and pyrosequencing

Genomic DNA of subjects was extracted with MagBead blood DNA kit purchased from CWbiotech (Beijing, P.R. China). We did bisulfite conversion on 0.5 μg genome DNA using the EZ DNA methylation kit (ZYMO Research) according to the manufacturer's instructions. The PCR pyrosequencing method to quantitate LINE-1 and AhRR promoter methylation was described previously [22,23]. After purification of PCR products using Sepharose beads on PyroMark Vacuum Prep Workstation (Qiagen), pyrosequencing was performed using the PyroMark Q96 ID System (Qiagen) according to the manufacturer's protocol. PCR and pyrosequencing primers of LINE-1 and AhRR are given in Appendix A, Table A1. The built-in controls were used to verify bisulfite conversion efficiency. The degree of methylation was expressed as the proportion of cytosines that were 5-methylated (%5mC). We measured DNA methylation levels at multiple CpG sites (four CpG site for LINE-1 and three CpG sites for AhRR) and calculated mean methylation values for each gene. We tested each marker in two replicates and used their average in the statistical analyses.

2.4. Statistical analysis

Statistical analysis was done using SAS 9.4 software. For our statistical calculations, results that were lower than the LOD of the methodology were expressed in values corresponding to the LOD divided by the square root of 2. Normal distribution test was examined using the Shapiro-Wilk normality test. Variables not fitting the normal distribution were compared using non-parametric tests. Chi-square tests were used to compare the frequencies of variable between the coke oven and non-coke oven groups. The data were presented as median and range. We also stratified the workers by the quartiles (25th percentile and 75th percentile) of PAH metabolites into low exposure (< 25th percentile), middle exposure (≥25th percentile but < 75th percentile), high exposure (≥75th percentile) subgroups. Workers belonging to low exposure were used as the reference group, and the multivariate logistic regression models with adjustment for confounders were used to investigate the associations. The dose response relationships between methylation of target gene and risk factor were also explored using test of linear trend and spline analysis. Differences considered statistically significant for P values were < 0.05.

3. Results

3.1. Main characteristics and PAHs exposure of study subjects

The characteristics of the coke oven workers and non-coke oven workers are summarized in Table 1. There were no differences in drinking, education degree and heating mode between coke oven workers and non-coke oven workers ($P > 0.05$), but the distribution of smoking was not different in two groups ($P < 0.05$). The median of age and employment year of subjects in the coke oven workers were significantly lower than the non-coke oven workers ($P < 0.05$). The distribution of males in the coke oven workers were significantly more than those in non-coke oven workers (95.1% vs. 71.0%, $P < 0.001$). The results also manifest the concentrations of each PAHs metabolite and ΣOH-PAHs metabolites in urine were significantly elevated in coke oven workers ($P < 0.001$). The airborne PAHs were monitored in working sites for the exposed group (top-, side- and bottom-ovens and operation room in the coke oven plant) and three working sites for the control group. The results showed he median values of the sum of eight carcinogenic PAHs and BaP in exposed sites were significant higher than control sites. This data were published in another paper [24].

3.2. Levels of LINE-1 and AhRR methylation among study subjects

The Spearman correlation coefficients (r) between mean methylation values (%5-methylcytosine) of four CpG sites and each site range from 0.448 to 0.811 ($P < 0.001$) in LINE-1, and the Spearman correlation coefficients (r) between mean methylation values of three CpG sites and each site range from 0.801 to 0.946 in AhRR ($P < 0.001$). So