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Specific long non-coding RNAs response to occupational PAHs exposure in coke oven workers

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ABSTRACT

To explore whether the alteration of lncRNA expression is correlated with polycyclic aromatic hydrocarbons (PAHs) exposure and DNA damage, we examined PAHs external and internal exposure, DNA damage and lncRNAs (HOTAIR, MALAT1, TUG1 and GAS5) expression in peripheral blood lymphocytes (PBLCs) of 150 male coke oven workers and 60 non-PAHs exposure workers. We found the expression of HOTAIR, MALAT1, and TUG1 were enhanced in PBLCs of coke oven workers and positively correlated with the levels of external PAHs exposure (adjusted $P_{trend} < 0.001$ for HOTAIR and MALAT1, adjusted $P_{trend} = 0.006$ for TUG1). However, only HOTAIR and MALAT1 were significantly associated with the level of internal PAHs exposure (urinary 1-hydroxypyrene) with adjusted $\beta = 0.298$, $P = 0.024$ for HOTAIR and $\beta = 0.090$, $P = 0.034$ for MALAT1. In addition, the degree of DNA damage was positively associated with MALAT1 and HOTAIR expression in PBLCs of all subjects (adjusted $\beta = 0.024$, $P = 0.002$ for HOTAIR and $\beta = 0.007$, $P = 0.003$ for MALAT1). Moreover, we revealed that the global histone 3 lysine 27 trimethylation (H3K27me3) modification was positively associated with the degree of genetic damage ($\beta = 0.061$, $P < 0.001$) and the increase of HOTAIR expression ($\beta = 0.385$, $P = 0.018$). Taken together, our findings suggest that altered HOTAIR and MALAT1 expression might be involved in response to PAHs-induced DNA damage.

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1. Introduction

PAHs are ubiquitous occupational and environmental contaminants, which have been classified as human genotoxicants and carcinogens [15,51]. Epidemiological studies have demonstrated that long-term exposure to PAHs links to high incidence of lung cancer in coke oven workers [5]. As a complex disease, the molecular etiology of cancer includes both genetic modifications and epigenetic aberrations. In genetics, DNA damage, oncogene activation and inactivation of tumor suppressor gene are believed to play important roles in PAHs-induced carcinogenesis [16]. In epigenetics, aberrant DNA methylation, histone modification and miRNA expression patterns have currently emerged as important mechanisms that contribute to PAHs-induced genotoxicity and carcinogenicity [6,7,24,32,46]. Long non-coding RNAs (lncRNA) is one of the essential epigenetic regulators. The specificity of lncRNA expression is now recognized as important epigenetic marks that confers lncRNAs with great potential as biomarkers for health risk assessment [29]. Accumulating evidence demonstrate that lncRNAs play critical roles not only in physiological processes of normal cells, but also in the development of many kinds of human diseases [10,29,33,36,43,45,48]. Recent studies found that alteration of lncRNA expression was involved in induction of several

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; PBLCs, peripheral blood lymphocytes; 1-OHP, 1-hydroxypyrene; lncRNAs, long non-coding RNAs; HOTAIR, HOX transcript antisense RNA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; TUG1, taurine up-regulated 1; GAS5, growth arrest-specific 5; H2K27me3, histone 3 lysine 27 trimethylation.

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chemicals-induced genotoxicity and cell malignant transformation, indicating the aberrant lncRNA changes might be promising biomarkers for risk prediction of environmental exposure [3,9,13,26,31,47]. However, how ambient PAHs exposure affects lncRNA expression and whether it is involved in development of adverse health effects have not been defined.

In this study, four candidate lncRNAs, HOTAIR, MALAT1, TUG1 and GAS5 that have been reported to be associated with DNA damage and cancer development [8,12,14,17,21,22,28,30,34,37–40,49,50] were selected to address the biological significance of lncRNAs. Our findings reveal that the altered HOTAIR and MALAT1 expression could be the sensitive biomarkers that indicate the PAHs exposure and PAHs-induced DNA damage.

2. Materials and methods

2.1. Study population and sample collection

In this study, 150 PAHs-exposure workers in coking plant and 60 non-PAHs exposure workers in hot-rolling mill from Ben Xi Iron and Steel Group Cooperation in Liaoning Province, China were recruited. Those workers who had suffered from acute infectious diseases, chronic diseases, long-term drug use, or exposed to mutagenic agents (such as X-ray radiation) within 2 months were excluded. Basic information of each subject was collected by a structured epidemiologic questionnaire including demographic information, educational level, smoking history, alcohol consumption, occupational exposure history, personal medical history, and grilled food intake. Additionally, 15 mL urine was collected for urinary 1-hydroxypyrene (1-OHP) detection and 5 mL of venous blood were drawn in an EDTA-Na_2 containing tube for comets assay and PBLCs isolation. PBLCs were isolated by using a standardized Ficoll-Hypaque gradient procedure in less than 3 h after the blood samples were obtained. All samples were kept at −80 °C before analysis. The protocol was approved by Research Ethic Committee of School of Public Health, Sun Yat-sen University, and informed consent was obtained from each participant.

2.2. Urine 1-OHP detection

5 mL of 4-day shift-end urine were collected and the measurement of 1-hydroxypyrene (1-OHP) were carried out according to the method described previously [19]. The level of urinary 1-OHP was detected by high-pressure liquid chromatography (HPLC) equipped with a fluorescence spectrophotometer and normalized by urinary creatinine (Cr) and presented as microgram per gram creatinine. The detection limit was 0.14 μg/L urine (signal/noise = 3). Measurements below the limit of detection (LOD) were replaced with LOD/$\sqrt{2}$.

2.3. Alkaline comets assay

The comet assay was performed using method described by Li et al. [24] and Singh et al. [35]. Comet assay was performed using the fresh blood sample according to the protocol. In order to minimize the variation, we placed the same numbers of samples collected from different groups (control, bottom, side and top groups) in one slide (CometSlide HT, Trevigen, USA). Analyses of the images were performed using Comet Assay Software Project-1.2.2 (University of Wroclaw, Poland). Olive tail moment (OTM) was selected as the parameter to indicate the degree of DNA damage.

2.4. RNA isolation and qRT-PCR

Total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, USA) and quantitative reverse transcription was carried out using TOYOBO RT-qPCR Kit (TOYOBO, Tokyo, Japan). The mRNA expression was detected using the SYBR Green Real time PCR Master Mix-Plus (TOYOBO, Tokyo, Japan) with the standard program

Table 1
General characteristics, PAHs exposure levels, lncRNA expression and biomarkers in PAH-exposed workers and controls.

	Controls (n = 60)	PAHs-exposed workers (n = 150)	P-value
General characteristic			
Age (years)	46.38 ± 8.97	42.20 ± 7.38	**0.002**[a]
Coke history(years)	–	16.29 ± 7.75	–
Smokers [n (%)]	39 (65.00)	97 (64.67)	0.963[b]
Smoking age (years)	15.39 ± 14.42	12.73 ± 11.42	0.208**a**
Drinkers [n (%)]	38 (63.00)	88 (58.67)	0.533[b]
Education (years)			0.662[b]
≤9	30 (50.00)	70 (46.67)	
>9	30 (50.00)	80 (53.33)	
BMI	24.93 ± 3.88	24.68 ± 2.95	0.657**a**
Ethic (Han/others)	58 (96.67)	120 (80.00)	**0.002**[b]
Grill food consumption [n (%)]	0 (0.00)	50 (66.67)	**<0.001**[b]
Internal exposure biomarker			
Urinary 1-OHP (μg/g creatinine)	7.09 (4.81,10.00)	43.70 (12.99,136.18)	**<0.001**[c]
Lnc RNA expression (−ΔCT) [d]			
HOTAIR	−13.21 (−11.80,−14.37)	−11.79 (−10.88,−12.81)	**<0.001**[c]
TUG1	−5.02 (−4.61,−5.32)	−4.20 (−3.48,−5.00)	**<0.001**[c]
MALAT1	−0.82 (−0.37,−1.27)	0.15 (0.74,−0.46)	**<0.001**[c]
GAS5	0.72 (1.23,0.29)	1.02 (1.43,0.50)	**0.495**[c]
DNA damage			
Olive tail moment	3.27 (0.00,5.54)	40.76 (4.86,53.61)	**<0.001**[c]
Histone modification			
H3K27me3 (%)	9.63 ± 3.82	12.50 ± 7.15	**0.030**[c]

Statistical significant indexes are in bold.
[a] Two-sides Student t test.
[b] Two-sides chi-square test.
[c] Multivariate covariance analysis with adjustment for BMI, age, smoke status, drinking status, grill food consumption, education, and ethics.
[d] The $\Delta CT = CT_{target} - CT_{GAPDH} - \Delta CT_{batchbalance}$.

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