Overexpression of miR-221 in peripheral blood lymphocytes in petrol station attendants: A population based cross-sectional study in southern China

Dalin Hua, Xiaowu Peng, Yungang Liu, Wenjuan Zhang, Xiaochun Peng, Huanwen Tang, Jianhui Yuan, Zhiliang Zhu, Jianping Yang

Department of Toxicology, Guangdong Provincial Key Laboratory of Tropical Disease Research, School of Public Health and Tropical Medicine, Southern Medical University, 1023 S. Shatai Road, Guangzhou 510515, China.

Department of Environment and Health, South China Institute of Environmental Sciences, Ministry of Environmental Protection, 7 S. East Yuancun Road, Guangzhou 510655, China.

Department of Toxicology, School of Public Health, Guangdong Medical College, 1 S. Xincheng Road, Science Park of Songshanhu Lake, Dongguan 523808, China.

Department of Toxicology, Shenzhen Center for Disease Control and Prevention, 8 S. Longyuan Road, Shenzhen 518055, China.

Department of Occupational Health, Baoan Center for Disease Control and Prevention, 116 S. Longjing Road, Shenzhen 518101, China.

Abstract

Benzene is a recognized environmental leukemogen, however, the mechanisms for its carcinogenesis have not been fully elucidated. Recently, miR-221, a suggested oncogene involved in a number of malignancies, has been detected with elevated expression levels in blood cells of patients with leukemia. To explore whether benzene exposure has an effect on the expression of miR-221, a population based cross-sectional study was conducted in southern China, with 97 petrol station attendants as the exposure group and 103 general residents as the control group. Plasma benzene was analyzed by using GC-MS. miR-221 in peripheral blood lymphocytes were measured by qRT-PCR and the ΔCt value for each sample was calculated by normalizing the Ct value for miR-221 with U6 RNA (i.e., ΔCt = Ct_{miR-221} − Ct_{U6}). Potential confounding factors were taken into account. Pearson correlation, univariate and multivariate logistic regression were performed in statistical analysis. The results showed that the air concentrations of benzene were significantly higher in petrol stations than in control sites (P < 0.05); The levels of benzene and miR-221 in exposure group were both significantly higher than in control group (P < 0.05) and there was a significant positive correlation between the two indexes (r = 0.851, P < 0.05); An association between benzene levels and the ΔCt values for miR-221 was identified by univariate and multivariate logistic analysis (OR 0.274; 95%CI 0.117, 0.396). Our investigation indicates that benzene exposure may be related to elevated miR-221 expression in human lymphocytes.

Keywords: miR-221, Lymphocyte, Benzene, Toxicology, Mechanisms

1. Introduction

MI RNAs, as non-coding and short chain RNA molecules (19–25 nucleotides), have the ability to bind to the 3' untranslated region
(3’UTR) of target genes complementarily and thus result in mRNA translation repression or accelerated mRNA degradation [Shi et al., 2008; Jeong, 2014; Guo et al., 2010; Gramantieri et al., 2009; Undi et al., 2013], by which various biological functions, such as signal transduction, cell development, differentiation, proliferation, apoptosis, reaction to stresses and carcinogenesis, can be regulated [Jeong, 2014; Undi et al., 2013; Machová Poláková et al., 2011; Frencelli et al., 2010; Aldaz et al., 2013; Gimenes-Teixeira et al., 2013]. Derelegation of miRNAs plays important roles in cancer development [Shi et al., 2008; Jeong, 2014; Fornari et al., 2008; le Sage et al., 2007]. For example, in chronic myeloid leukemia (CML), derelegation of miR-221, miR-150, miR-20a and miR-17 results in dysfunctions of their target genes, which encode proteins involved in cell cycle and growth regulation as well as several key signaling pathways related to the development of CML [Machová Poláková et al., 2011]. Especially, miRNAs can function as oncogenes which is associated with initiation, promotion and progression of various cancers [Son et al., 2015; Garzon et al., 2009] and a specific miRNA may interfere with the expression of a set of genes involved in multiple cellular pathways [Xu et al., 2015; Lewis et al., 2005]. So, miRNAs have been suggested as biomarkers for the diagnosis and prognosis of some cancers, and as potential targets for the biologic therapy [Gramantieri et al., 2009; Lan et al., 2015].

Benzene is a ubiquitous air contaminant and a recognized environmental leukemogen, yet the mechanisms for its carcinogenesis have not been fully elucidated [Snyder, 2012]. Recently, miR-221, which has been suggested to be an oncogene, was frequently found to be overexpressed in patients with various types of leukemia and be associated with poor prognosis [Gimenes-Teixeira et al., 2013; Fornari et al., 2008; le Sage et al., 2007; Galardi et al., 2007; Medina et al., 2008; Rommer et al., 2013]. We are interested in whether the expression of miR-221 is altered in humans following benzene exposure, which has not been reported. In this study, we have carried out a population based cross-sectional study in southern China, in which the levels of miR-221 expression in the peripheral blood lymphocytes (PBL) of healthy petrol station attendants (PSAs) and its relevance to the internal benzene exposure were analyzed.

2. Materials and methods

2.1. Study population

The study was carried out during the period from May 2011 to October 2014 in southern China. The studied population consisted of exposure and control groups, they worked an average of six days per week for an average of eight working hours per day in various petrol stations in southern China, all of whom had no history of cancers or other chronic diseases and had not been taking any medicines within at least the recent year before blood sample collection. The eligible exposure population was PSAs who had been working for at least more than 3 years at petrol stations. The control population was GRs recruited from the same cities, with no history of occupational exposure to petrol, organic solvent, pesticides, heavy metals, or any other genotoxic substances. The information of the participants was collected by trained interviewers using the questionnaires, including demographic data [e.g., age, gender, body mass index (BMI), education, etc.], lifestyle factors (focusing on alcohol consumption and smoking habit, the alcohol consumption is defined as the habit of “current alcohol intake”, including the dichotomic variables of “yes” or “no”, the smoking habit is defined as the “current smoking habit including the dichotomic variables of “yes” or “no”), medical history, and occupational factors [type of work, exposure age (years) and personal protective measures, etc.]. The distribution of the confounding factors was kept equal between the two groups of participants. Finally, the exposure group consisted of 97 eligible PSAs (59 males and 38 females; mean age 27.97 ± 6.72 years) selected from different petrol stations in southern China, and the control group consisted of 103 eligible GRs (61 male and 42 female; mean age 28.11 ± 7.19 years) selected from residents who lived in the same cities. The research procedures for the recruitment and data collection were approved by the local ethical research committee and the purpose of the study was described in detail prior to obtaining written informed consent from each participant.

2.2. Blood and air sample collection

Five ml of peripheral venous blood from each selected participant in the exposure and control groups was collected at the end of work time by using single-use syringe, and sodium citrate anti-coagulation tubes were used to collect blood, with standard laboratory practice to ensure contamination-free collection. In order to avoid possible bias, the samples were labeled with the subject’s number, date and time of collection. Then the samples were stored and transported in a cooler with dry ice. Between 11:30 and 15:30 during week days, air samples in a breathing zone (1.4 m) at either petrol stations where PSAs were recruited from or ambient control sites where GRs were recruited from, were collected in SKC glass tubes (SKC Inc. U.S.A.) filled with charcoal, by using portable battery-operated pump with flow rate at 80 ml/min and 50-min sampling time. 48 air samples in various petrol stations in southern China were collected as the exposure group and 16 air samples in general resident areas were collected as the controls at the same time. Since benzene is much more toxic than the other chemicals present in the petrol as well (e.g., toluene and xylene), this research was focused on the effect of benzene.

2.3. Plasma and lymphocyte separation

Lymphocyte separation medium (Sigma—Aldrich) was used to achieve plasma and lymphocyte separation according to the manufacturer’s instructions. The separated plasma was stored in sterilized Eppendorf tubes, numbered and kept in a refrigerator at −80 °C for detection of the concentration of benzene using GC/MS. The separated lymphocytes were washed with PBS for three times, and then resuspended in DMSO, numbered, and stored at 2 °C–8 °C for miR-221 analysis.

2.4. Measurement of concentrations of benzene in plasma samples

In this study, plasma benzene was selected as the internal exposure marker of benzene. Prior to the selection of plasma for benzene detection, fifteen peripheral venous blood samples and the corresponding plasma samples were both measured for the concentrations of benzene, and the results showed a good correlation between the two sets of data (r = 0.903, p < 0.01). Therefore, the use of plasma benzene as a biomarker of internal benzene exposure is feasible. For detecting the concentrations of benzene in plasma samples, GC/MS analysis was conducted on instrument of QP2010plus (Shimadzu, Japan.) equipped with automatic sampler of AOC-20i+ (Shimadzu, Japan) and an Rtx-Wax (30 m × 0.32 mm × 0.25 μm) column (Restek Company, U.S.A.). Helium was used as the carrier gas at a constant flow rate (0.9 ml/min). Splitless mode was used. An injection volume of 1 μl was run with instant splitless time 0.2 min. A direct analysis was performed after headspace extraction. The inlet temperature was set at 200 °C. The column temperature was initially maintained at 35 °C for 5 min, and then increased to 100 °C at a rate of 15 °C/min. The ion source temperature was set to 200 °C. The MS quadrupole...
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