RESEARCH ARTICLE

Impact of AhR, CYP1A1 and GSTM1 Genetic Polymorphisms on TP53 R273G Mutations in Individuals Exposed to Polycyclic Aromatic Hydrocarbons

Meili Gao[1], Yongfei Li[2], Xiaochang Xue[3], Jiangang Long[1], Lan Chen[4], Walayat Shah[5], Yu Kong[1]*

Abstract

This study was to undertaken to investigate the impacts of AhR, CYP1A1, GSTM1 genetic polymorphisms on the R273G mutation in exon 8 of the tumor suppressor p53 gene (TP53) among polycyclic aromatic hydrocarbons (PAHs) exposed to coke-oven workers. One hundred thirteen workers exposed to PAH and 82 control workers were recruited. We genotyped for polymorphisms in the AhR, CYP1A1, GSTM1, and TP53 R273G mutation in blood by PCR methods, and determined the levels of 1-hydroxypyrene as PAH exposure marker in urine using the high pressure liquid chromatography assay. We found that the distribution of alcohol users and the urinary excretion of 1-OHP in the exposed workers were significantly higher than that of the control workers (p=0.004, p<0.001, respectively). Significant differences were observed in the p53 genotype distributions of smoking subjects (p=0.01, 95%CI: 1.23-6.01) and PAH exposure (p=0.008, 95%CI: 1.24-4.48), respectively. Further, significant differences were observed in the p53 exon 8 mutations for the genetic polymorphisms of Lys/Arg for AhR (p=0.02, 95%CI: 0.70-15.86), Val/Val for CYP1A1 (p=0.04, 95%CI: 0.98-19.09) and null for GSTM1 (p=0.02, 95%CI: 1.19-6.26), respectively. Our findings indicated that polymorphisms of PAH metabolic genes, such as AhR, CYP1A1, GSTM1 polymorphisms may interact with p53 genetic variants and may contribute to PAH related cancers.

Asian Pac J Cancer Prev, **15 (6)**, 2699-2705

Introduction

PAHs are mediated by aryl hydrocarbon receptors (AhR), which are ligand- activated transcriptional factors that play an important role in benzo[a]pyrene-induced carcinogenesis (Shimizu et al., 2000). AhR translocates into the nucleus to interact with xenobiotic responsive elements after binding with benzo[a]pyrene (B[a]P). This results the up-regulation of phase I and II enzymes, such as cytochrome P450 (CYP450) and glutathione S-transferases (GST), which are also involved in the metabolism of PAHs (Jiang et al., 2005). AhR polymorphisms will cause higher binding affinities or more functionally efficient which makes their carriers more sensitive to environment compounds exposure (Maier et al., 1998; Ramadoss et al., 2005; Gu et al., 2012). Kawajiri and his colleagues firstly found one of the polymorphisms in human AhR, G1661A (Arg 554 Lys; rs2066853). This polymorphism has been the most commonly studied in the AhR gene polymorphism since then (Kawajiri et al., 1995).

CYP450 and GST are two important genes which encode carcinogen metabolizing enzymes, and involved in the metabolism of carcinogens including PAHs. Cytochrome P450 1A1 (CYP1A1) is the member of subfamily 1 of the CYP gene super family. It play a key role in the catalysis of oxidative reactions and xenobiotics like B[a]P to carcinogenic reactive metabolites. The polymorphism of CYP1A1(BseMI) of TgC transition is located downstream of exon7 (m2 polymorphism). This polymorphism results in a substitution of a isoleucine (Ile) for a valine (val) mutation at amino acid residue 462 in the heme binding region of CYP1A1 protein (Chen et al., 2006; Giri et al., 2012). GST is a multigene family which includes five subgroups (i.e., α, π, μ, τ and ζ). The cytosolic enzyme glutathione S-transferase μ1 (GSTM1) is encoded by the GSTM1 gene. GSTM1 detoxifies activated forms of chemical carcinogens such as polyaromatic hydrocarbon epoxides. It has been reported that GSTM1 allele is lost in a subset of the general population (Eaton and Bammler, 1999). For example, this gene is deleted in about 50% of

*[1]Institute of Mitochondrial Biology and Medicine, Department of Biological Science and Engineering, [4]Center of Shared Experimental Facilities, The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, [2]School of Materials and Chemical Engineering, Xi'an Technological University, [3]State Key Laboratory of Cancer Biology, Department of Biopharmaceutics School of Pharmacy, Fourth Military Medical University, Xi'an, Shaanxi, China, [5]Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan *For correspondence: gaomeili@mail.xjtu.edu.cn*

Caucasians, with a reported variation of from 38 to 65% (Board et al., 1990). Therefore, association between the inherited absence of the GSTM1 gene (the GSTM1 null genotype) and the higher risk to the toxic effects as well as its influence on various biomarkers of exposure has been widely investigated (Sram and Binkova, 2000; Chen et al., 2007; Moretti et al., 2007).

Generally, CYP450 enzymes metabolized PAHs to form the ultimate carcinogenic diol epoxides which form covalent adducts with DNA, especially r7, t8-dihydroxy-t-9, 10 epoxy-7, 8, 9, 10- tetrahydro- benzo[a] pyrene (BPDE) (Cavalieri and Rogan, 1995; Smith et al., 2000; Shimada and Fujii-Kuriyama, 2004). Tumor suppresser p53 gene (TP53) is one of the most commonly mutated genes observed in human tumors. PAHs have been found to cause p53 mutations. It has been reported that codons 157, 248, and 273 of the p53 gene were attacked by BPDE to form guanine adducts in normal human bronchial epithelial cells (Denissenko et al., 1996; Smith et al., 2000; Lai et al., 2006). These mutational hotspots of p53 were reported to be associated with more aggressive malignancies and could confer novel phenotypes in vivo, including an increased metastatic capacity and resistance to chemotherapies (Garritano et al., 2013). Several studies indicated the high frequency at codon 273 (C to G) in exon 8 of the TP53 gene, CGU to GGU, leading to a substitution of an arginine for a serine (R273G mutation) in PAH compounds adduction (Gonzalez et al., 2000; Smith et al., 2000).

As described above, AhR, CYP450 and GST are involved in the metabolism of PAHs carcinogen. TP53 gene may be the metabolites of PAHs attacked gene. However, no study has investigated these genotypes interaction among PAHs exposed workers. Hence, in the present study, we investigated the impact of some select genetic polymorphisms of AhR, CYP1A1, GSTM1 genes on the R273G mutation in exon 8 TP53 gene among PAHs exposed coke-oven workers.

Materials and Methods

Study subjects

The 113 coke-oven workers and 82 non–coke-oven workers who were all males and worked in the same steel company in northern China were studied in this paper. These 113 coke-oven workers were in active service at the time of the study and employed for at least 6 months which were recruited as the exposed group. The 82 non–coke-oven workers were staff members of the offices and hospitals of the same steel company, and served as the control group. The workers who exposed to known mutagenic agents, including radiotherapy and chemotherapy in the last 3 months, were excluded. Questionnaires were administered by trained interviewers to collect information on demographic information, including age, employment time, smoking and alcohol habits. Individuals who had smoked for 3 months were considered as smokers. Those who drank more than twice a week in the last six months were classified as drinkers. In the morning, 5 ml fasting venous blood and 10 ml urine samples was collected from each subject for further analysis. The study was approved by the Ethics Committee of Xi'an Jiaotong Medical College and was performed in accordance with the Helsinki Declaration. All test persons gave their written informed consent prior to their participation in the investigation.

Analysis of 1-hydroxypyrene in urine

Urine 1-hydroxypyrene was measured by the method described by Jongeneelen and Anzion (1990) and Siwińska et al. (2004). Briefly, aliquots of 10 ml urine samples was enzymatically deconjugated and transferred to primed C18 octadecyl cartridges (Beckman, USA). Then the samples were washed with 10 ml of water, and eluted with 9 ml of methanol. The components of the elutae was subjected to a high pressure liquid chromatography of the Waters Alliance 2695 (Waters, USA) with the XAqua C18, 150×4.1 mm column. A fluorescence detector of F1000 (Hitachi, Japan) was quantitatively assayed 1-hydroxypyrene concentration. The wavelengths of excitation and emission were 229 nm and 400 nm, respectively. The concentrations of 1-hydroxypyrene were normalised to urinary creatinine. Urinary creatinine concentrations were assayed by a standard colorimetric method with the picric acid reaction and absorption at 520 nm (Baselt, 1980).

Genotyping for AhR, GSTM1, CYP1A1 and p53 gene mutation

Genomic DNA was extracted from 1 mL of whole blood from subjects by a Promega Genomic DNA Purification Kit (Promega Corp., Shanghai, China) and stored at 4°C. The AhR gene polymorphism of the G1661A (Arg554Lys) was assayed according to the published protocol (Zhang et al., 2002). Briefly, primers of the AhR allele–specific were used to get a 333bp product. The amplification of a 493-bp product of β–actin gene was used as an internal control. For quality control, 10% of DNA samples were genotyped again and the concordance of two sets of genotyping data was 100%. The genotypes of GSTM1 were analyzed by a multiplex PCR as described by Zhong et al. (1993) and Wiencke et al. (1995). The absence of a GSTM1-specific 230bp indicated the GSTM1-null genotype when the positive control of β-actin-specific 157bp product was present detected simultaneously in one reaction. The m2 polymorphism of the CYP1A1 gene was determined by PCR-RFLP (Bufalo et al., 2006). The primers for m2 were 5'-TTCCACCCGTTGCAGCAGGATAGC C-3'and 5'-CTGTCTCCCTCTGGTTACAGGAAG-3', which generate a 204bp fragment. The amplified products were visualized in ethidium bromide-stained gels. The restriction enzyme BseMI was used to identify the m2 polymorphism. The wild-type allele has two fragments of 149bp and 55bp. The homozygote variant generates a single band representing the entire 204bp fragment and the heterozygote variant presents the three bands, according to the manufacturer's protocol (Dingguo Life Sciences). The restricted products were analyzed by electrophoresis in 3% agarose gels containing ethidium bromide. RFLP results of CYP1A1 m2 were confirmed by DNA sequencing of PCR products. We also looked for the presence of point mutation in exon 8 of TP53 in the plasma

DNA by PCR-SSCP analysis described by Gonzalez et al. (2000). The primers used for amplification of the exon 8 were: 5-GGGACAGGTAGGACCTGATTTCCTT and 5-ATCTGAAGGCATAACTGCACCCTTGG. Briefly, the amplified products were denatured by mixing with 15ul of denaturing stop solution and the allelic band intensity on the gels was detected after electrophoresis, the amplified DNA fragments were further used for direct DNA sequencing with the dNTP method.

Statistical analysis

All analyses were carried out using the Statistical Package for Social Sciences (SPSS12.0). The frequencies of categorical variables, such as smoking and drinking status, p53 gene mutations, AhR, CYP1A1 and GSTM1 genotypes between groups were compared by chi-square test. Student's t tests were used to compare the age, employment time, PAH exposure between control and exposed groups. All statistical tests were two-sided with a significant level of $p<0.05$.

Results

General characteristics

The relevant characteristics of exposed workers and controls are shown in Table 1. No differences were found in the distributions of age, employment time, and smoking status between control and exposed groups. On the other hand, the distribution of alcohol users in the exposed workers were significantly higher than that of the control workers ($p=0.004$). The urinary excretion of 1-OHP as the PAH exposure marker was significantly different ($p<0.001$) between control and exposed subjects.

The distributions of TP53, AhR, CYP1A1, GSTM1 genotypes

Table 2 and Table 3 show the number and percent distributions of the subjects by TP53, AhR, CYP1A1, GSTM1 genotypes. Table 2 shows the distribution of TP53 exon 8 mutations and age, employment time, smoking status, drinking status as well as PAH exposure. The percent distributions of mutated TP53 were higher of age, employment time, smoking status, drinking status by chi-square test in exposed group than in control group, though not significant differences (Table 2). The frequency for mutated TP53 was higher of PAH exposure in exposed group (45, 39.82%) than in control group (18, 21.95%). Significant differences were observed in

Table 1. Demographic Data by Exposure Situations among Coke-oven and Control Workers

Variables	Control group (n=82)	Exposed group (n=113)	p
Age [years, mean(SD)]	33.3±5.6	34.5±5.3	0.21[b]
Employment time [years, mean, (SD)]	13.5±2.3	13.9±2.8	0.29[b]
Current smokers, yes (%)	44(53.7%)	68(60.2%)	0.36[c]
Alcohol users, yes (%)	21(25.6%)	51(45.1%)	0.004[c]
PAH exposure[a]	0.58±0.12	4.90±1.15	<0.001[b]

[a]PAH exposure evaluated by urinary excretion of 1-pyrenol (μmol/mol creatinine); [b]Student's t test for comparison between two groups; [c]Chi-square test for comparison between two groups

smoking status, drinking status as well as PAH exposure. The percent distributions of mutated TP53 were higher of age, employment time, smoking status, drinking status by chi-square test in exposed group than in control group, though not significant differences (Table 2). The frequency for mutated TP53 was higher of PAH exposure in exposed group (45, 39.82%) than in control group (18, 21.95%). Significant differences were observed in the TP53 genotype distributions of smoking subjects ($p=0.01$,

Table 2. Stratification Analysis of p53 Exon 8 Mutations in the Subjects by Smoking, Drinking and PAH Exposure Situations

Variables	Control group		Exposed group		OR[a]	p
	Wild type p53	Mutated p53	Wild type p53	Mutated p53		
Smoking						
Yes	30 (36.59)	14 (17.07)	30 (26.55)	38 (33.63)	2.71[b]	0.001
No	34 (41.46)	4 (4.88)	38 (33.63)	7 (6.19)	1.57	0.500
Alcohol						
Yes	15 (18.29)	6 (7.31)	26 (23.00)	25 (22.12)	2.4	0.110
No	49 (59.76)	12 (14.63)	42 (37.17)	20 (17.70)	1.94	0.110
PAH exposure						
	64 (78.05)	18 (21.95)	68 (60.18)	45 (39.82)	2.35[c]	0.008

*Data are presented as number (%); [a]Chi-square test for comparison between two groups; [b]95%CI, 1.23-6.01; [c]95%CI, 1.24-4.48

Table 3. Stratification Analysis of AhR, CYP1A1 and GSTM1 Genotypes in the Subjects by Exposure Situations

	Control group n (%)	Exposed group n (%)	OR[a]	p[b]
AhR				
Arg/Arg	33 (40.2)	41 (36.3)	1.18	0.57
Lys/Arg	36 (43.9)	52 (46.0)	0.92	0.77
Lys/Lys	13 (15.9)	20 (17.7)	0.88	0.73
Lys/Arg- Lys/Lys	49 (59.8)	72 (63.7)	0.85	0.57
CYP1A1				
Ile/Ile	32 (39.0)	42 (37.2)	1.08	0.79
Ile/Val	35 (42.7)	53 46.9)	0.84	0.56
Val/Val	15 (18.3)	18 (15.9)	1.18	0.66
Ile/Val-Val/Val	50 (61.0)	71 (62.8)	0.92	0.79
GSTM1				
Non-null	35 (42.7)	47 (41.6)	1.05	0.88
Null	47 (57.3)	66 (58.4)	0.96	0.88

*Data are presented as number (%); [a]Compared between the two groups by chi-square test after adjustment for age, work site, and pack-years of cigarettes smoking; [b]Asymp. Sig (2-sided)

Table 4. Comparisons of the p53 Gene Mutations in Subgroups Stratified by Genotypes of AhR, CYP1A1, and GSTM1 in the Control and Exposed Groups

Variables	Control group		Exposed group		OR[a]	p[b]
	Wild type p53	Mutated p53	Wild type p53	Mutated p53		
AhR						
Arg/Arg	25 (75.8[f])	8 (24.2[f])	28 (68.3[f])	13 (31.7[f])	1.45	0.48
Lys/Arg	29 (80.56)	7 (19.44)	30 (57.69)	22 (42.31)	3.04[c]	0.02
Lys/Lys	10 (76.9)	3 (23.1)	10 (50.0)	10 (50.0)	3.333	0.122
CYP1A1						
Ile/Ile	27 (84.3)	5 (15.7)	29 (69.0)	13 (31.0)	2.42	0.13
Ile/Val	26 (74.3)	9 (25.7)	32 (60.38)	21 (39.62)	1.9	0.18
Val/Val	11 (73.3)	4 (26.7)	7 (38.9)	11 (61.1)	4.32[d]	0.04
GSTM1						
Non-null	28 (80.0)	7 (20.0)	32 (68.1)	15 (31.9)	1.88	0.23
Null	36 (76.6)	11 (23.40)	36 (54.6)	30 (45.4)	2.73[e]	0.02

*Data are presented as number (%); [a]Chi-square test for comparison between two groups; [b]Asymp. Sig. (2-sided); [c]95%CI, 0.70-15.86; [d]95%CI, 0.98-19.09; [e]95%CI, 1.19-6.26; [f]Show the percent of the number of wild type p53 or mutated p53 in the number of AhR, CYP1A1 and GSTM1 polymorphisms (Table 3)

the TP53 genotype distributions of smoking subjects (p=0.01, 95%CI, 1.23-6.01) and PAH exposure (p=0.008, 95%CI, 1.24-4.48), respectively. The percent frequency for the AhR Lys^{554}, CYP1A1 val variant genotype and GSTM1 null genotype was slight higher in exposed group (63.7%, 62.8%, 58.4%, respectively) than in control subjects (59.8%, 61.0%, 57.3%, respectively), though not significant differences.

Data are presented as number (%): [a]compared between the two groups by chi-square test after adjustment for age, work site, and pack-years of cigarettes smoking; [b]Asymp. Sig (2-sided).

Correlation between TP53 gene mutation and AhR, CYP1A1 and GSTM1 genotypes

The distribution of TP53 exon 8 mutations in subgroups stratified by genotypes of AhR, CYP1A1 and GSTM1 was shown in Table 4 and Figure 1. No significant differences were found for the distribution of the AhR, CYP1A1, GSTM1 genotypes except the genetic polymorphisms of Lys/Arg for AhR, Val/Val for CYP1A1 and null for GSTM1 (Table 4) between control and exposed groups. The distributions of the genetic polymorphisms of Lys/Arg for AhR, Val/Val for CYP1A1 and null for GSTM1 were higher in exposed (42.31%, 61.1% and 45.4%, respectively) than in control group (19.44%, 26.7% and 23.4%, respectively). Significant differences (p=0.02, 95%CI, 0.70-15.86; p=0.04, 95%CI, 0.98-19.09; p=0.02, 95%CI, 1.19-6.26, respectively) were observed in the TP53 exon 8 mutations for the genetic polymorphisms of Lys/Arg for AhR, Val/Val for CYP1A1 and null for GSTM1.

The percent distributions of wild type p53 and mutated p53 with AhR, CYP1A1 and GSTM1genotypes in the exposed and control groups are shown in Figure 1.The frequency of mutated p53 in control group with Lys/Arg for AhR, Val/Val for CYP1A1 and null for GSTM1 was 7(8.54%), 4(4.88%), 11(13.41%), respectively. The distribution number and percent of mutated p53 in exposed group with Lys/Arg for AhR, Val/Val for CYP1A1 and null for GSTM1 was 22(19.47%), 11(9.73%), 30(26.55%), respectively. In addition, the relative lower of the distribution of wild type p53 in exposed group than in control group was observed. Similarly, the relative higher distribution of mutated p53 in exposed group when compared to control group was also observed in Figure 1.

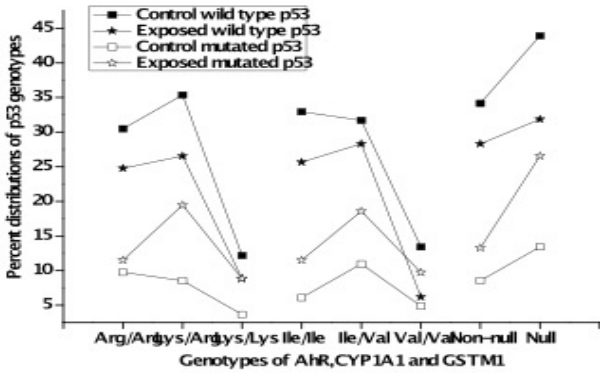

Figure 1. Distributions of Wild Type p53 and Mutated p53 with AhR, CYP1A1 and GSTM1 Genotypes in the Exposed and Control Groups

Discussion

The purpose of this study was to determine the distributions of R273G mutation in TP53 exon 8, AhR, CYP1A1 and GSTM1 genotypes and to see the effects of PAH on these gene variants, on the one hand, and to see the impacts of AhR, CYP1A1 and GSTM1 genotypes on the R273G mutation in TP53 exon 8 in PAH exposed coke oven workers, on the other hand.

PAH are ubiquitous in ambient air and in a certain occupational environment, particularly in the coke production. Epidemiologic studies indicate that occupational exposure to PAH was well correlated with the levels of PAH metabolites such as 1-OHP in urine. 1-OHP is a major metabolite of pyrene that has been shown to be a good marker for total PAH exposure and is thought to reflect PAH activation (Nerurkar et al., 2000; Castaño-Vinyals et al., 2004; Bin et al., 2008). Data from the present study also showed that levels of urinary 1-OHP differed significantly according to exposure to PAHs among coke oven workers. This is in consistent with occupational studies on PAH-exposed persons, including coke oven workers (Ovrebo et al., 1998; van Delft et al., 2001; Bin et al., 2008). The results were higher than the investigations performed in West European coke oven workers which revealed lower levels of 1-OHP, not exceeding in average the concentration of 2 μmol/mol creatinine (Ovrebo et al., 1998; van Delft et al., 2001; Marczynski et al., 2002).

Our data demonstrated that the frequency of R273G mutation in exon 8 of TP53 was significantly higher (p=0.01, OR=2.71, 95%CI: 1.23-6.01) by the smokers and PAH exposure (p=0.008, OR=2.35, 95%CI: 1.24-4.48) for the coke oven workers. In fact, several studies reported that the high frequency at codon 273 (C to G) in exon 8 of the TP53 gene was related to a higher risk of PAH exposure (Smith et al., 2000; Park et al., 2008) as well as lung cancer development (Gonzalez et al., 2000; Hussain et al., 2001). With respect to tobacco smoke, this is not contradictory to the assumption that cigarette smoke has relative high PAHs and other carcinogens which result in high expression levels of p53 and increased p53 mutations (Yao et al., 2005; Gao et al., 2011). There was a significantly higher incidence of alcohol users (Table 1) in exposed group than in control group. However, no significant increase (p=0.11) in the incidence of TP53 mutation was observed in alcohol users (Table 2). In agree with our results, Lazarus et al. reported that no increase in the incidence of p53 mutation was observed in tobacco users who drank alcohol in oral cavity squamous cell carcinomas (Lazarus et al., 1996). On the contrary, according to the reports described by Hsieh et al., the effect of alcohol on the incidence of p53 mutations was still statistically significant (RR=2.24; 95%CI, 1.21-4.15) after adjustment for cigarette smoking and betel quid (BQ) chewing (Hsieh et al., 2001). This raises the possibility that alcohol drinking could enhance the mutagenic effects of p53.

Studies have indicated that AhR can be activated by a wide range of classes of compounds including PAHs. The Arg554Lys polymorphism of AhR gene (rs2066853)

correlates with altered expression of the CYP1A1 detoxification enzyme. Generally, GSTM1 can detoxify reactive metabolites of benzo(a)pyrene and other PAHs (Smart and Daly, 2000; Chen et al., 2006; Giri et al., 2012). Here, we found that the frequency of variants in the AhR Lys^{554} and CYP1A1 val variant genotype and GSTM1 null genotype showed slight higher in exposed group (63.7%, 62.8%, 58.4%, respectively) than in control subjects (59.8%, 61.0%, 57.3%, respectively) on urinary 1-OHP excretion, though did not show significant differences. This is in agreement with the findings of AhR Lys^{554}, CYP1A1 val variant genotype, GSTM1 null genotype and 1-OHP levels in PAH exposure population. For example, in a Caucasian population, induced CYP1A1 activity was found to be higher in subjects with the AhR Lys 554 variant genotype than those with AhR Arg 554/ Arg554genotype (Smart et al., 2000). In addition, the results were similar as the association of these gene variants and PAH-DNA adducts levels. Gu et al. studied the six tag SNPs of the AHR gene, rs2066853, rs1476080, rs2158041, rs2106728, rs713150 and rs6960165, only two of rs2066853 and rs2158041 variants, were associated with PAH-DNA adduct levels in glioma tissue (Gu et al., 2012). Nerurkar et al. (2000) found the association of an increased PAH activation with the CYP1A1 variant alleles at the levels of exposure observed in occupationally exposed workers and in smokers. Several studies have showed that mean levels of 1-OHP levels were higher in individuals with the GSTM1 null genotype than those with a GSTM1 positive genotype (Alexandrie et al., 2000). However, conflicting evidence also exists. Some studies show that the R554K change does not alter the ability of AhR to regulate CYP1A1- or CYP1B1-driven transcription (Celius and Matthews, 2010). Reports have indicated that the coke-oven workers without the GSTM1 allele did not have a higher risk of having high benzo[a] pyrene diolepoxide–DNA adduct levels compared with individuals with the GSTM1 non-null genotype (van Delft et al., 2001).

As the rs2066853 polymorphism is located in the transactivation domain and leads to a nonsynonymous amino acid substitution in the AhR protein, which has been demonstrated to influence its function (Gu et al., 2012). CYP1A1 is the most potently induced gene following AHR activation. The homozygous deletion of GSTM1 leads to the loss of GSTM1 functions (Nerurkar et al., 2000; Chen et al., 2006; Moretti et al., 2007). Therefore, the results indicated that the functions of AhR, CYP1A1 and GSTM1 were more impaired or modified with higher PAH exposure. No difference among mutant variants of AhR, CYP1A1 and GSTM1 in 1-OHP levels were detected in this study. Chen et al. (2007) reports have also indicated that no influence was found in the association between urinary 1-OHP and such as CYP1A1 polymorphisms (Chen et al., 2007). Simultaneously, GST is mainly excreted as glucuronide conjugate, GST activity is not thought to be directly linked to the metabolism of 1-OHP. This may be the possible reason for GST null genotype in PAH exposure subjects (Strickland et al. 1997). Further, the results might be attributable to the fact that, the lower levels of PAH exposure (Ovrebo et al., 1998; van Delft et al., 2001; Marczynski et al., 2002), exposure of the general population to PAHs may be too intermittent to be detectable with a cross-sectional design. Another possibility is that our study lacked the power to reveal the effect of AhR, CYP1A1 and GSTM polymorphisms on 1-OHP levels in the exposed group, or that these enzymes have tissue specificity for the target organs in PAH exposure subjects.

Generally, activation of the AhR by PAH result in the up-regulation of phase I and II enzymes, such as cytochrome P450 and glutathione S-transferases, which are also involved in the metabolism of PAHs. Further, the metabolism of PAHs induces DNA damage and carcinogenesis which could be modulated by genetic variation (Shimizu et al., 2000; Jiang et al., 2005; Ramadoss et al., 2005; Chen et al., 2006). In this study, significant difference (p=0.02, 95%CI, 0.70-15.86) was observed in the R273G mutation in p53 exon 8 for the genetic polymorphisms of Lys/Arg for AhR. The result suggests that R273G mutation in p53 exon 8 is modulated by AhR gene polymorphisms. In addition, Yang et al. (1999) found that DBA/2 genotype at the TP53 locus were relatively resistant to tetrachlorodibenzo-p-dioxin toxicity, and this resistance was additive with resistance associated with the AhR locus (Yang et al., 1999). Nakatsuru et al. (2004) reported that bromodeoxyuridine-labeling index and accumulation of p53 protein in epidermal cells of AhR+/+ mice were 8- and 33-fold higher than those of AhR-/- mice, respectively (Nakatsuru et al., 2004). These findings implied that p53 gene variants might be affected with AhR gene polymorphisms. It is also corresponding with the previously described AhR role in the metabolism of PAHs. Significant associations between CYP1A1 val homozygous (OR=4.32, p=0.04) and GSTM1 null genotype (OR=2.73, p=0.02) in mutated TP53 R273G in this study were observed in this study. Bartsch et al. (2000) found that GSTM1*0/0 deficient genotype was increased risks of p53 mutations, DNA adducts and other markers of effect in tobacco related cancers (Bartsch et al., 2000). It was also shown that the p53 gene is more frequently mutated in lung cancer patients with the CYP1A1 Val/Val genotype according to the reports described by Kawajirii et al. (1996) and Przygodzki et al. (1998). However, Rusin and his colleagues have not detected the similar relationship in non-small-cell lung cancer from an environmentally polluted region of Poland (Rusin et al., 1999). Although the GSTM1null genotype has been correlated with increased risk for p53mutations, this association has not been observed in all studies. For example, a significant association was not observed between the GSTM1 null genotype and the prevalence of p53 mutations in lung tumors (Kawajiri et al., 1996). Further, PAH exposure results in the relative higher distribution (Figure 1) of mutated p53 with AhR, CYP1A1 and GSTM1 genotypes when compared to control group in the present study. The results implied that susceptible AhR, CYP1A1 and GSTM genotypes were at remarkably high risk of having a mutation of the R273G mutation in TP53 gene in PAH exposed subjects. As described above, the published reports as well as our findings are not in consistent with each other. Therefore, the genotypes of

AhR, CYP1A1 and GSTM on the R273G mutation in p53 exon 8 need be further studied in the next study.

In conclusion, this study showed a significant higher for the distribution of alcohol users and the urinary excretion of 1-OHP levels between the control and exposed groups. Smoking and PAH were also significantly affect the distributions of the TP53 exon 8 of R273G mutation. Our data indicated that variants of Lys/Arg for AhR, Val/Val for CYP1A1 and null for GSTM1 were significantly and positively associated with R273G mutation. The alteration of PAH metabolic genes polymorphisms may interact with p53 genetic variants and further contribute to tumorigenesis in relation to PAHs exposed subjects.

Acknowledgements

The research was supported by the Fundamental Research Funds for the Central Universities (2012jdhz48) and by the Shaanxi Postdoctoral Science Foundation funded project (18420024).

References

Alexandrie AK, Warholm M, Carstensen U, et al (2000). CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure. *Carcinogenesis*, **21**, 669-76.

Bartsch H, Nair U, Risch A, et al (2000). Genetic polymorphism of CYP genes, alone or in combination as a risk modifier of tobacco related cancers. *Cancer Epidemiol Biomark Prev*, **9**, 3-28.

Baselt RC (1980). Biological monitoring methods for industrial chemicals. Davis, CA: Biomedical Publications, 207-11.

Bin P, Leng S, Cheng J, et al (2008). Association of aryl hydrocarbon receptor gene polymorphisms and urinary 1-hydroxypyrene in polycyclic aromatic hydrocarbon-exposed workers. *Cancer Epidemiol Biomarkers Prev*, **17**, 1702-08.

Board P, Coggan M, Johnston P, et al (1990). Genetic heterogeneity of the human glutathione transferases: a complex of gene families. *Pharmacol Ther*, **48**, 357-69.

Bufalo NE, Leite JL, Guilhen AC, et al (2006). Assumpcao and laura s ward. Smoking and susceptibility to thyroid cancer: an inverse association with CYP1A1 allelic variants. *Endocr Relat Cancer*, **13**, 1185-93.

Castano-Vinyals G, D'Errico A, Malats N, et al (2004). Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution. *Occup Environ Med*, **61**, 12.

Cavalieri EL, Rogan EG (1995). Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica*, **25**, 677-88.

Celius T, Matthews J (2010). Functional analysis of six human aryl hydrocarbon receptor variants in human breast cancer and mouse hepatoma cell lines. *Toxicology*, **277**, 59-65.

Chen Y, Bai Y, Yuan J, et al (2006). Associationof polymorphisms in AhR, CYP1A1, GSTM1, and GSTT1 genes with levels of DNA damage in peripheral blood lymphocytes among coke-oven workers. *Cancer Epidemiol Biomarkers Prev* **15**, 1703-07.

Chen B, HuY, Jin T, et al (2007). The influence of metabolic gene polymorphis ms on urinary 1-hydroxypyrene concentration s in Chinese coke oven workers. *Sci Total Environ*, **381**, 38-46.

Denissenko MF, Pao A, Tang M, et al (1996). Preferential formation of benzo (a) pyrene adducts at lung cancer mutational hotspots in P53. *Science*, **274**, 430-2.

Eaton DL, Bammler TK (1999). Concise review of the glutathione s-transferases and their significance to toxicology. *Toxicol Sci*, **49**, 156-64.

Gao M, Li Y, Sun YL, et al (2011). A common carcinogen benzo[a]pyrene causes p53 overexpression in mouse cervix via DNA damage. *Mutat Res*, **724**, 69-75.

Garritano S, Inga A, Gemignani F, et al (2013). More targets, more pathways and more clues for mutant p53. *Oncogenesis*, **2**, 54.

Giri SK, Yadav A, Kumar A, et al (2012). CYP1A1 gene polymorphisms: modulator of genetic damage in coal-tar workers. *Asian Pac J Cancer Prev*, **13**, 3409-16.

Gonzalez R, Silva JM, Sanchez A, et al (2000). Microsatellite alterations and TP53 mutations in plasma DNA of smallcell lung cancer patients: followup study and prognostic significance. *Oncology*, **11**, 1097-104.

Gu A, Ji G, Jiang T, et al (2012). Contributions of aryl hydrocarbon receptor genetic variants to the risk of glioma and PAH-DNA adducts. *Toxicol Sci*, **128**, 357-64.

Hsieh LL, Wang PF, Chen IH, et al (2001). Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. *Carcinogenesis*, **22**, 1497-503.

Hussain SP, Amstad P, Raja K, et al (2001). Mutability of p53 hotspot codons to benzo (a) pyrene diol epoxide (BPDE) and the frequency of p53 mutations in nontumorous human lung. *Cancer Res*, **61**, 6350-5.

Jiang H, Shen YM, Quinn AM, et al (2005). Competingroles of cytochrome P450 1A1/1B1 and aldo-keto reductase 1A1 in the metabolic activation of (+/-)-7, 8-dihydroxy-7,8-dihydro-benzo[a]pyrene in human bronchoal-veolar cell extracts. *Chem Res Toxicol*, **18**, 365-74.

Jongeneelen FJ, Anzion RBM (1990). Analyses of hazardous substances in biological materials. *Deutsche Forschungsgemeinschaft*, **3**, 151-69.

Kawajiri K, Eguchi H, Nakachi K, et al (1996). Association of CYP1A1germ line polymorphisms with mutations of the p53 gene in lung cancer. *Cancer Res*, **56**, 72-6.

Kawajiri K, Watanabe J, Eguchi H, et al (1995). Polymorphisms of human Ah receptor gene are not involved in lung cancer. *Pharmacogenetics*, **5**, 151-8.

Lai TJ, Tsai YY, Cheng YW, et al (2006). An association between BPDE-like DNA adduct levels and p53 gene mutation in pterygium. *Mol Vis*, **12**, 1687-91.

Lazarus P, Stern J, Zwiebel N, et al (1996). Relationship between p53 mutation incidence in oral cavity squamous cell carcinomas and patient tobacco use. *Carcinogenesis*, **17**, 733-9

Maier A, Micka J, Miller K, et al (1998). Aromatic hydrocarbon receptor polymorphism: development of new methods to correlate genotype with phenotype. *Environ Health Perspect*, **106**, 421-6.

Marczynski B, Rihs HP, Rossbach B, et al (2002). Analysis of 8-oxo-7,8-dihydro-2 -deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. *Carcinogenesis*, **23**, 273-81.

Moretti M, Dell'Omo M, Villarini M, et al (2007). Primary DNA damage and genetic polymorphisms for CYP1A1, EPHX and GSTM1 in workers at a graphite electrode manufacturing plant. *BMC Public Health*, **7**, 270.

Nakatsuru Y, Wakabayashi K, Fujii-Kuriyama Y, et al (2004). Dibenzo (A,L) pyrene-induced genotoxic and carcinogenic responses are dramatically suppressed in aryl hydrocarbon receptor-deficient mice. *Int J Cancer*, **112**, 179-83.

Nerurkar PV, Okinaka L, Aoki C, et al (2000). CYP1A1,

GSTM1, and GSTP1 genetic polymorphisms and urinary 1-hydroxypyrene excretion in non-occupationally exposed individuals. *Cancer Epidemiol Biomarkers Prev*, **9**, 1119-22.

Ovrebo S, Ryberg D, Haugen A, et al (1998). Glutathione S-transferase M1 and P1 genotypes and urinary excretion of 1-hydroxypyrene in coke oven workers. *Sci Total Environ*, **220**, 25-31.

Park JH, Gelhaus S, Vedantam S, et al (2008). The pattern of p53 mutations caused by PAH o-quinones is driven by 8-oxo-dGuo formation while the spectrum of mutations is determined by biological selection for dominance. *Chem Res Toxicol*, **21**,1039-49.

Przygodzki RM, Bennett WP, Guinee MD, et al (1998). p53 mutation spectrum in relation to GSTM1, CYP1A1 and CYP2E1 in surgically treated patients with non-small cell lung cancer. *Pharmacogenetics*, **8**, 503-11.

Ramadoss P, Marcus C, Perdew GH (2005). Role of the aryl hydro-carbon receptor in drug metabolism. *Expert Opin Drug Metab Toxicol*, **1**, 9-21.

Rusin M, Butkiewicz D, Malusecka E, et al (1999). Molecular epidemiological study of non-small-cell lung cancer from an environmentally polluted region of Poland. *Br J Cancer*, **80**, 1445-52.

Shimada T, Fujii-Kuriyama Y (2004). Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer Sci*, **95**, 1-6.

Shimizu Y, Nakatsuru Y, Ichinose M, et al (2000). Benzo[a] pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA*, **97**, 779-82.

Siwinska E, Mielzynska D, Kapka L (2004). Association between urinary 1-hydroxypyrene and genotoxic effects in coke oven workers. *Occup Environ Med*, **61**, 10.

Smart J, Daly AK (2000). Variation in induced CYP1A1 levels: relationship to CYP1A1, Ah receptor and GSTM1 polymorphisms. *Pharmacogenetics*, 10, 11-24.

Smith LE, Denissenko MF, Bennett WP, et al (2000). Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. *J Natl Cancer Inst*, **92**, 803-11.

Sram RJ, Binkova B (2000). Molecular epidemiology studies on occupational and environmental exposure to mutagens and carcinogens, 1997-1999. *Environ Health Perspect*, **108**, 57-70.

Strickland PT, Kang D, Bowman ED, et al (1994). Identification of 1-hydroxypyrene glucuronide as a major metabolite in human urine by synchronous fluorescence spectroscopy and gas chromatography-mass spectrometry. *Carcinogenesis*, **15**, 483-7.

van Delft JH, Steenwinkel MS, van Asten JG, et al (2001). Biological monitoring the exposure to polycyclic aromatic hydrocarbons of coke oven workers in relation to smoking and genetic polymorphisms for GSTM1 and GSTT1. *Ann Occup Hyg*, **45**, 395-408.

Wiencke JK, Pemble S, Ketterer B, et al (1995). Gene deletion of glutathione s-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol Biomarkers Prev*, **4**, 253-9.

Yang AL, Smith AG, Akhtar R, et al (1999). Low levels of p53 are associated with resistance to tetrachlorodibenzo-p-dioxin toxicity in DBA/2 mice. *Pharmacogenetics*, **9**, 183-8.

Yao R, Wang Y, D'Agostini F, et al (2005). K-ras mutations in lung tumors from p53 mutant mice exposed to cigarette smoke. *Exp Lung Res*, **31**, 271-81.

Zhang DS, Lin GF, Ma QW, et al (2002). Nonassociation of aryl hydrocarbon receptor genotypes with susceptibility to bladder cancer in Shanghai population. *Acta Pharmacol Sin*, **23**, 188-92.

Zhong S, Wyllie AH, Barnes D, et al (1993). Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. *Carcinogenesis*, **14**, 1821-4.