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Correlation between Gene Expression of Aryl Hydrocarbon Receptor (*AhR*), Hydrocarbon Receptor Nuclear Translocator (*Arnt*), Cytochromes P4501A1 (*CYP1A1*) and 1B1 (*CYP1B1*), and Inducibility of *CYP1A1* and *CYP1B1* in Human Lymphocytes

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The relationships between gene expression of aryl hydrocarbon receptor (AhR), aryl hydrocarbon receptor nuclear translocator (Arnt), cytochromes P4501A1 (CYP1A1), 1B1 (CYP1B1), *CYP1A1*, and the inducibility of *CYP1A1* and *CYP1B1* were determined in 32 cultivated human lymphocytes. Cytochrome P450 induction was performed by incubating lymphocytes with benzanthracene. The relative gene expression levels were determined by quantitative real-time RT-PCR assay. We found that gender is an important confounding factor for gene expression in cultivated lymphocytes. *AhR*, *CYP1A1* and *CYP1B1* levels in noninduced lymphocytes were significantly higher in female nonsmokers than in male nonsmokers ($p < 0.05$). Nevertheless, *CYP1A1* and *CYP1B1* inducibility was lower in female nonsmokers. *CYP1A1* inducibility was higher in male smokers than in male nonsmokers ($p < 0.05$). After controlling for gender and cigarette smoking, *AhR* levels positively correlated with *CYP1B1* levels and *CYP1A1* inducibility ($p < 0.01$ and $p = 0.03$, respectively). *Arnt* levels also correlated with *CYP1B1* levels in induced lymphocytes ($p < 0.01$). However, *AhR* levels were negatively correlated with *CYP1B1* inducibility. These data indicate that *AhR* expression associates with individual variation of *CYP1A1* inducibility and *CYP1B1* expression in cultivated lymphocytes. Furthermore, gender and cigarette smoking are important confounding factors for gene expression levels in cultivated lymphocytes.

Key Words: **aryl hydrocarbon receptor; hydrocarbon receptor nuclear translocator; cytochrome P4501A1; cytochrome P4501B1.**

Several studies have indicated that drug-metabolizing enzyme activities are highly variable in the human population (Nebert, 1991). Some drug-metabolizing enzymes are responsible for metabolic activation of environmental carcinogens. Thus, individual variation in metabolic activation or detoxification of environmental carcinogens partially explains the host susceptibility to chemical-induced toxicity (Nebert, 1991; Perera, 1996).

The cytochrome P450-1 family is one of the major cytochrome P450 families involved in xenobiotic metabolism. One of the well known examples is cytochrome P4501A1 (CYP1A1), which has been shown to participate in metabolic activation of polycyclic aromatic hydrocarbons (PAHs) (Shimada *et al.*, 1992). PAHs are some of the major carcinogens found in the environment as pollutants. Heavy exposure to PAH-contaminated air pollutants has been associated with the increased risk of lung cancer (Boffetta *et al.*, 1997). It has been demonstrated that CYP1A1 activity and expression are inducible by PAHs through activation of aryl hydrocarbon receptor (AhR) (Whitlock, 1999). CYP1A1 inducibility measured in cultivated lymphocytes has correlated well with that measured in lung tissue explants (Jacquet *et al.*, 1997). Therefore, it was suggested that CYP1A1 inducibility is a susceptibility marker for PAH-induced lung carcinogenesis (Kellermann *et al.*, 1973; Kiyohara *et al.*, 1998). Although the association between CYP1A1 inducibility and lung cancer risk is still controversial (Kellermann *et al.*, 1973; Prasad *et al.*, 1979; Stucker *et al.*, 2000; Ward *et al.*, 1978), CYP1A1 inducibility by PAHs is variable in the human population (Kellermann *et al.*, 1973). Little information has been available on the molecular mechanism of variation in CYP1A1 inducibility. *CYP1A1* genetic polymorphisms have been demonstrated to correlate with CYP1A1 inducibility (Kiyohara *et al.*, 1996, 1998). However, the genotypes with high CYP1A1 inducibility are extremely rare in some ethnic groups (Hirvonen *et al.*, 1992; Tefre *et al.*, 1991; Xu *et al.*, 1996), and other factors should contribute to individual variation in CYP1A1 inducibility (Smart and Daly, 2000).

Differential *CYP1A1* inducibility was also reported in different strains of mice and was correlated with PAH-induced carcinogenesis in these mice (Nebert, 1989). The mice with high *CYP1A1* inducibility had a higher AhR ligand-binding capacity. The PAHs are AhR ligands. The liganded AhR trans-

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locates from the cytosol to the nuclei, heterodimerizes with AhR nuclear translocator (Arnt), binds to the cognate enhancer sequence, and subsequently transactivates gene expression of *CYP1A1* and cytochrome P4501B1 (CYP1B1) (Whitlock, 1999). CYP1B1 was also involved in metabolic activation of PAHs (Shimada *et al.*, 1996). Constitutive expression and induction of *CYP1B1* has been found in lung cancer cell lines and lymphocytes (Chang *et al.*, 1999; Spencer *et al.*, 1999). However, no research has been done to investigate the individual variation in *CYP1B1* expression and inducibility in human population. It has been demonstrated that AhR activation is required for PAH-induced toxicity (Nebert, 1989; Shimizu *et al.*, 2000). The study by Hayashi *et al.* (1994) demonstrated individual differences in the mRNA levels of AhR and Arnt in human liver and lung tissues. The mRNA levels of *CYP1A1* from the blood of healthy subjects correlated with that of *AhR* and *Arnt* (Hayashi *et al.*, 1994). Nevertheless, it is still uncertain whether the differential expression of *AhR* and *Arnt* contributes to the interindividual variation in *CYP1A1* inducibility.

CYP1A1 inducibility by PAHs can be measured in cultivated lymphocytes. Aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD) assays were utilized to determine CYP1A1 activity in cultivated lymphocytes (Jacquet *et al.*, 1997; Kellermann *et al.*, 1973). However, a few problems exist in these assays. For example, the AHH assay is not specific to CYP1A1 activity and the EROD assay is not sensitive enough to detect basal levels of CYP1A1 activity (Jacquet *et al.*, 1997). It is worthwhile to explore more accurate and specific quantitative assays for CYP1A1 induction in lymphocytes. Since it is well known that the PAHs induce CYP1A1 activity at the transcription level (Whitlock, 1999), quantification of *CYP1A1* mRNA levels is a potential tool for measuring *CYP1A1* inducibility in lymphocytes. In the present study, we developed the quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) assay to quantify the relative mRNA levels in cultivated lymphocytes. We propose that the expression levels of *AhR* and *Arnt* contribute to the differences in *CYP1A1* and *CYP1B1* inducibility in humans. Utilizing this technique, we investigated whether the expression levels of *AhR* and *Arnt* correlated with inducibility of *CYP1A1* and *CYP1B1* in cultivated peripheral lymphocytes isolated from 32 healthy subjects. Other factors, such as gender and cigarette smoking, were also considered. Information generated from this study will be helpful in the elucidation on the mechanism of interindividual variation in *CYP1A1* and *CYP1B1* inducibility and expression. These techniques can be further utilized to assess the roles of AhR, Arnt, CYP1A1, and CYP1B1 in lung carcinogenesis in the future.

MATERIALS AND METHODS

Study subjects. A total of 32 healthy subjects were recruited in this study, including 10 male nonsmokers, 12 male smokers, and 10 female nonsmokers. The average age was 24 ± 3 years. All of the donors were Taiwan citizens and were interviewed for cigarette smoking status. The Chung Shan Medical University Hospital Institutional Review Board approved this project. Samples were collected in the morning. Approximately 20 ml of venous blood was collected from each donor with heparinized tubes.

TABLE 1
Primer Sequences and Concentrations for Quantitative Real-time RT-PCR

Genes	Sequences	Concentration (nM)
β-actin	5′-TCATGAAGTGTGACGTGGACATC-3′	100
	5′-CAGGAGGAGCAATGATCTTGATCT-3′	
AhR	5′-ACATCACCTACGCCAGTCGC-3′	400
	5′-TCTATGCCGCTTGGAAGGAT-3′	
Arnt	5′-GCTGCTGCCTACCCTAGTCTCA-3′	200
	5′-GCTGCTCGTGTCTGGAATTGT-3′	
CYP1A1	5′-CACCATCCCCCACAGCAC-3	100
	5′-ACAAAGACACAACGCCCCTT-3′	
CYP1B1	5′-GCTGCAGTGGCTGCTCCT-3′	100
	5′-CCCACGACCTGATCCAATTCT-3′	

Lymphocytes isolation and culture. Lymphocytes were isolated from fresh blood samples using Ficoll-Paque gradients according to the manufacturer's instructions. Isolated lymphocytes were washed with phosphate-buffered saline and cell numbers were determined. Cell concentrations were adjusted to 10^6/ml in RPMI medium containing 5 μg/ml PHA, 10% fetal bovine serum, and 0.1% dimethylsulfoxide (DMSO) or 12 μM benzanthracene (BA). Cell cultures were maintained at 37°C, 5% CO_2, and 95% relative humidity until harvest. After harvest, viable cell numbers in each culture were determined with trypan blue exclusion method. Total RNAs of cells and tissues were prepared using TRI_{ZOL}® Reagent (Life Technologies, Rockville, MD) and the phenol-chloroform extraction method. Isolated RNA was stored at –70°C until analysis.

Quantitative real-time RT-PCR assay. Total RNA of cells and tissues were measured using the TRI_{ZOL} Reagent (Life Technologies, Rockville, MD) and the phenol-chloroform extraction method. Synthesis of cDNA was done with 2 μg total RNA, 1 μg oligo dT primer and 20 nmol deoxynucleotide triphosphates using M-MLV Reverse Transcriptase (Promega, Madison, WI). Quantitative PCR was performed using the QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany) and analyzed on an ABI PRISM 7700 Sequence Detector System (Perkin-Elmer Applied Biosystems, Foster City, CA). Primers were chosen with the assistance of the computer program *Primer Express* (Perkin-Elmer Applied Biosystems). To avoid amplification of contaminating genomic DNA, one of the two primers was placed at the junction between two exons. The primer sequences and optimal concentrations of *AhR*, *Arnt*, *CYP1A1*, *CYP1B1*, and *β-actin* are listed in Table 1. The thermal cycling comprised an initial step at 50°C for 2 min, followed by a denaturation step at 95°C for 10 min, 40 cycles at 95°C for 15 s, and 40 cycles at 60°C for 1 min. Varying lengths of oligonucleotides produce dissociation peaks at different melting temperatures. Consequently, at the end of the PCR cycles, the PCR products were analyzed using the heat dissociation protocol to confirm that one single PCR product was detected by SYBR Green dye. Each data point was repeated four times. Quantitative values were obtained from the threshold PCR cycle number (Ct) at which the increase in signal associated with an exponential growth for PCR product starts to be detected. The relative mRNA levels in each sample were normalized to its *β-actin* content. The relative expression levels of the target gene: $2^{-\Delta Ct}$, $\Delta Ct = Ct_{target\ gene} - Ct_{\beta\text{-actin}}$.

Statistical analyses. Data analysis started with descriptive statistics of all important variables. Arithmetic means and standard deviation of each gene

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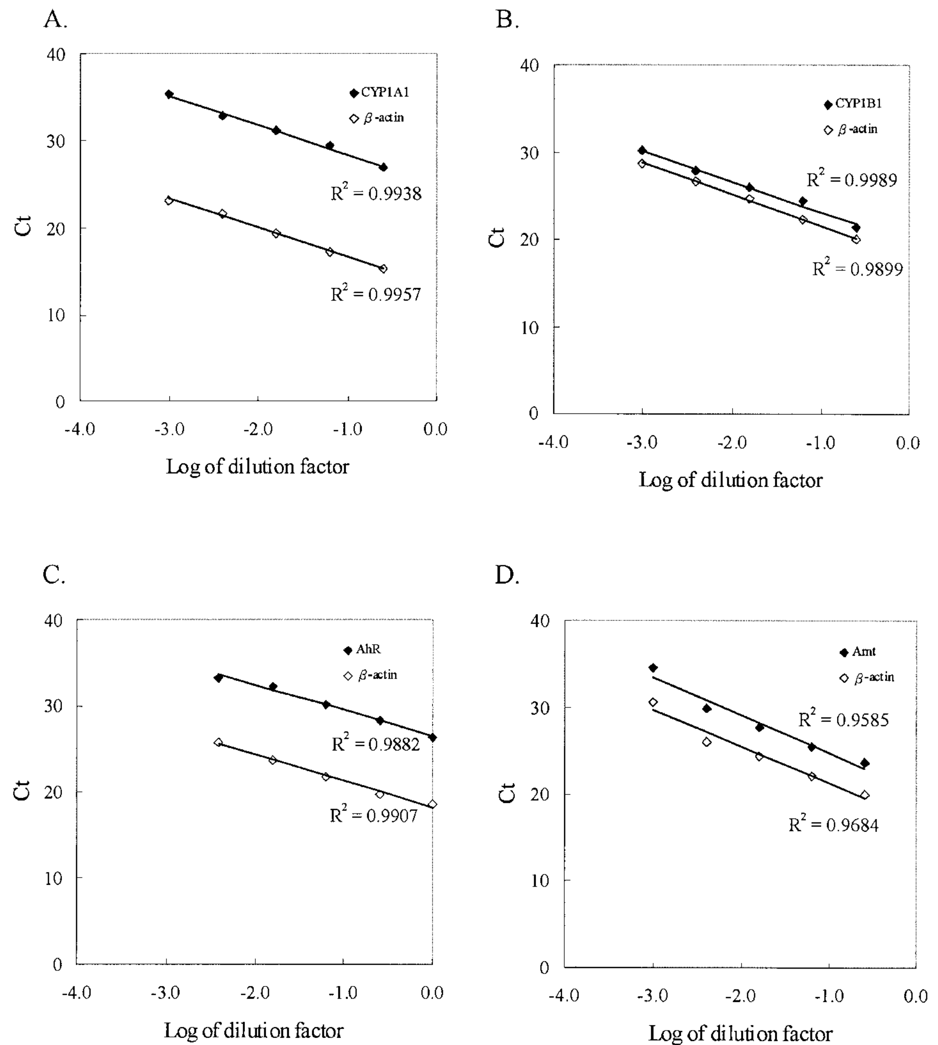

FIG. 1. Validation of the quantitative real-time RT-PCR method. The cDNA from human lung adenocarcinoma cells NCI-H1355 cells was serially diluted, amplified with β-actin primer and (A) CYP1A1, (B) CYP1B1, (C) AhR, or (D) Arnt primers, and detected by SYBR green with the quantitative real-time RT-PCR assay. The quantification data was presented with Ct. These data were fitted using linear regression analysis.

expression level and frequency of categorical variables were calculated. In order to normalize the distributions, *CYP1A1* and *CYP1B1* expression levels were transformed into natural logarithmic scale. Comparisons of gene expression levels between males and females, between smokers and nonsmokers, and between BA treatment and DMSO treatment, respectively, were done by Student's *t*-test. Pearson's correlation coefficient was used to assess the correlation between gene expression levels. Finally, the multiple linear regression models were applied to assess the association between *AhR* and *Arnt*, respectively, with *CYP1A1* and *CYP1B1* controlling for gender and smoking status. Potential interaction between *AhR* or *Arnt* and gender or smoking status was evaluated by adding the interaction terms *AhR**gender, *AhR**smoking, *Arnt**gender, and *Arnt**smoking, respectively, into the linear regression models. An alpha level of 0.05 was used for all statistical tests.

RESULTS

The linear range of the quantitative real-time RT-PCR assay was determined by amplifying serial dilutions of cDNA converted from lung cancer cell lines NCI-H1355. The standard curve was examined by amplifying 4-fold serial dilutions of the cDNA using primers for *AhR*, *Arnt*, *CYP1A1*, *CYP1B1*, and *β-actin*. The standard curve is plotted by the log of the template dilution fold versus the Ct (Fig. 1). The standard curve of *AhR*, *Arnt*, *CYP1A1*, and *CYP1B1* were all parallel to that of *β-actin*, which indicated that the amplification efficiency of *β-actin* and target genes was equal. The calculation for the relative gene expression levels is described in Materials and Methods.

The time course of gene expression in cultivated lymphocytes was determined after exposure to 12 μM BA for 0 to 3 days. Lymphocytes were isolated from four donors: two male smokers, one male nonsmoker and one female nonsmoker. As shown in Figure 2, *CYP1A1* levels were increased at day 1 and continued to increase within 3 days. Similarly, *CYP1B1* levels started to increase at day 2 and then kept increasing to day 3. *AhR* and *Arnt* levels remained consistent after BA treatment. Lymphocytes isolated from 2 of 4 subjects died on day 4 after BA treatment (data not shown). Therefore, we decided to measure the induction of *CYP1A1* and *CYP1B1* expression on day 3 in the following study. However, it is unclear why the basal *CYP1B1* expression, but not *AhR*, *Arnt*, or *CYP1A1*, kept

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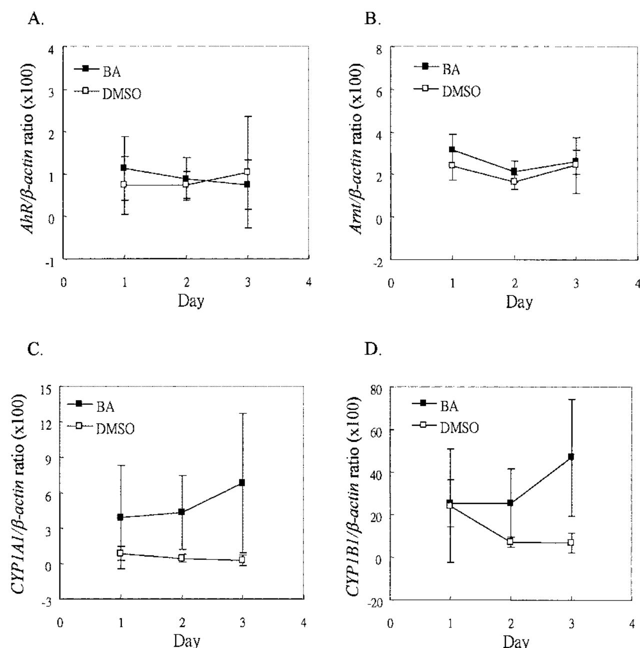

FIG. 2. Time course of gene expression levels in DMSO- and BA-treated lymphocytes. Peripheral lymphocytes isolated from 4 donors were cultivated in the presence of 0.1% DMSO or 12 μM BA for 1, 2, and 3 days. The relative gene expression levels of (A) *AhR*, (B) *Arnt*, (C) *CYP1A1*, and (D) *CYP1B1* were determined with the quantitative real-time RT-PCR assay. The relative gene expression levels were normalized with those of *β-actin* and calculated from $2^{-\Delta Ct}$, as described in Materials and Methods.

decreasing during 3 days of cultivation. We also compared gene expression levels in uncultured lymphocytes and 3-day cultivated lymphocytes. We found that *CYP1A1* levels were significantly lower in uncultivated lymphocytes than in cultivated lymphocytes (data not shown). However, *AhR*, *Arnt*, and *CYP1B1* levels in uncultivated lymphocytes were not significantly different from those in cultivated lymphocytes (data not shown).

Isolated peripheral lymphocytes were treated with 0.1% DMSO or 12 μM BA for 3 days and then harvested to quantify the relative gene-expression levels. Cell viability in DMSO- or BA-treated cells was, respectively, 85 or 75% (data not shown). The mRNAs of *AhR*, *Arnt*, *CYP1A1*, and *CYP1B1* were all detectable in lymphocytes (Table 2). *CYP1A1* and *CYP1B1* levels in DMSO-treated cells varied by ~200- and 100-fold, respectively. Individual variations of *AhR* and *Arnt* levels in DMSO-treated cells were, respectively, 50- and 13-fold. *CYP1A1* and *CYP1B1* levels were significantly increased after BA treatment, but *AhR* and *Arnt* levels were not significantly changed. The average induction folds of *CYP1A1* and *CYP1B1* were 45.86 and 4.73, respectively.

The effects of smoking and gender on gene expression levels were evaluated by analyzing log-transformed data with a Student's *t*-test. The nontransformed data was presented in Table 3. No significant difference in gene expression levels was found between male smokers and nonsmokers, but *CYP1A1* inducibility was significantly higher in smokers than in nonsmokers. *AhR*, *CYP1A1*, and *CYP1B1* levels in DMSO-treated cells from female nonsmokers were significantly higher than those from male nonsmokers. On the other hand, *CYP1A1* and *CYP1B1* inducibility was significantly higher in males than in females. However, *Arnt* levels did not differ by gender or smoking status. Nonparametric analysis (Mann-Whitney U-test) for untransformed data was consistent with the above results (data not shown).

TABLE 2
Gene Expression Levels and Inducibility of *AhR*, *Arnt*, *CYP1A1*, and *CYP1B1* in DMSO- and BA-treated Lymphocytes

	Treatment		
Genes	DMSO	BA	Inducibility
Ahr	2.03 ± 1.64[a]	1.51 ± 1.11	—
	(0.12 – 6.50)[b]	(0.11 – 3.63)	
Arnt	4.53 ± 3.12	4.84 ± 3.83	—
	(4.53 – 10.38)	(0.67 – 14.31)	
CYP1A1	0.26 ± 0.50	2.40 ± 3.69[c]	45.86 ± 61.59
	(0.01 – 2.07)	(0.33 – 21.46)	(0.24 – 282.09)
CYP1B1	22.00 ± 26.12	36.39 ± 25.89[c]	4.73 ± 5.92
	(0.97 – 115.74)	(4.79 – 104.07)	(0.22 – 23.02)

[a]Mean ± standard deviation of relative gene expression in the original scale.
[b]Range of relative gene expression in the original scale.
[c]Compared with DMSO-treated cells, $p < 0.05$, Student's *t*-test.

TABLE 3
Gene Expression Levels and Inducibility in Lymphocytes after Stratified by Gender and Cigarette Smoking Status

	Male		Female
	Smokers ($n = 12$)	Nonsmokers ($n = 10$)	Nonsmokers ($n = 10$)
DMSO-treated cells			
AhR	1.83 ± 1.38[a]	1.14 ± 0.72	3.16 ± 2.03[c]
Arnt	4.75 ± 2.78	5.05 ± 4.18	3.67 ± 2.37
CYP1A1	0.18 ± 0.47	0.08 ± 0.08	0.53 ± 0.67[c]
CYP1B1	22.69 ± 33.07	7.03 ± 4.72	36.15 ± 22.79[c]
BA-treated cells			
AhR	1.21 ± 0.99	1.38 ± 1.03	2.01 ± 1.24
Arnt	4.50 ± 3.66	3.92 ± 3.39	6.19 ± 4.44
CYP1A1	3.97 ± 5.72	1.65 ± 1.01	1.26 ± 0.70
CYP1B1	43.65 ± 29.52	33.52 ± 28.79	30.56 ± 17.24
Inducibility			
CYP1A1	87.77 ± 83.11[b]	31.31 ± 21.52	10.12 ± 2.75[c]
CYP1B1	5.76 ± 6.37	7.11 ± 7.00	1.12 ± 0.74[c]

[a]Mean ± standard deviation of gene expression or inducibility.
[b]Comparison between male nonsmokers and male smokers; $p < 0.05$, Student's *t*-test in natural logarithm-transformed scale.
[c]Comparison between male nonsmokers and female nonsmokers, $p < 0.05$, Student's *t*-test in natural logarithm-transformed scale.

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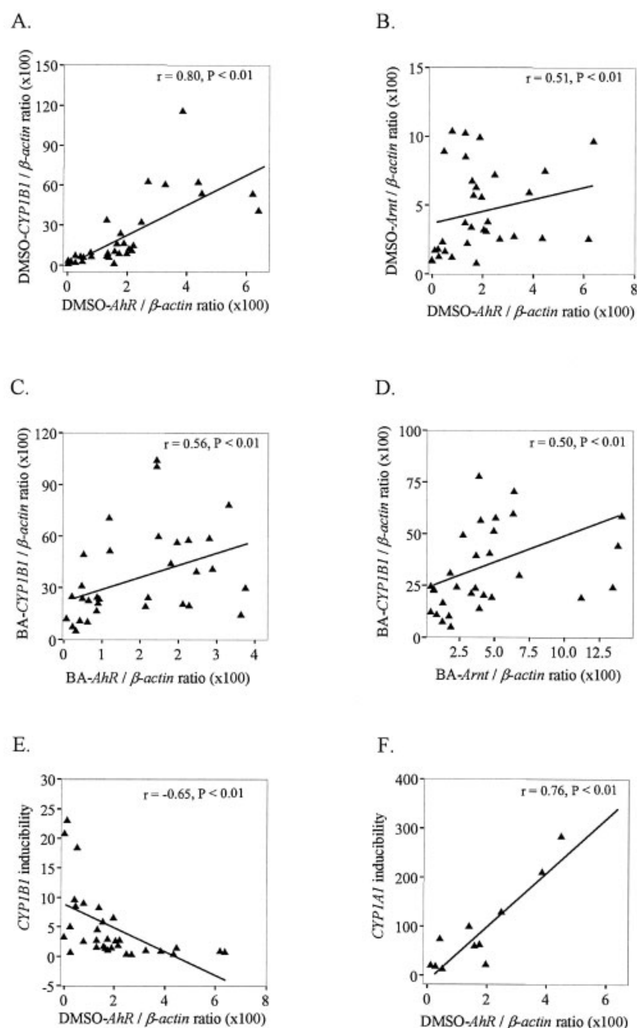

FIG. 3. Correlation of gene expression levels of *AhR*, *Arnt*, *CYP1A1*, *CYP1B1*, and inducibility of *CYP1A1* and *CYP1B1* in lymphocytes. Gene expression levels were normalized with those of *β-actin* and calculated from $2^{-\Delta Ct}$ as described in Materials and Methods. Inducibility was the fold change in gene expression levels of BA- versus DMSO-treated cells. These data were fitted using linear regression analysis. In DMSO-treated cells, *AhR* levels correlated with (A) *CYP1B1* and (B) *Arnt* levels. In BA-treated cells, *CYP1B1* levels correlated with (C) *AhR* and (D) *Arnt* levels. *AhR* levels in DMSO-treated cells correlated with (E) *CYP1B1* and (F) *CYP1A1* inducibility.

It is well known that AhR and Arnt regulate *CYP1A1* and *CYP1B1* gene expression. Therefore, we further investigated whether *AhR* and *Arnt* levels are associated with expression levels and the inducibility of *CYP1A1* and *CYP1B1*. Log-transformed data were analyzed with the Pearson correlation. We found that *CYP1B1* and *Arnt* levels positively correlated with *AhR* levels in DMSO-treated cells (Figs. 3A and 3B). In BA-treated cells, *AhR* and *Arnt* levels both correlated with *CYP1B1* levels (Figs. 3C and 3D). *CYP1B1* inducibility was negatively correlated with *AhR* levels in DMSO-treated cells (Fig. 3E). However, the correlation was not observed between *CYP1A1*, *Arnt*, and *AhR* (data not shown). When subjects were stratified according to smoking status, *CYP1A1* inducibility positively correlated with *AhR* levels in DMSO-treated cells from smokers (Fig. 3F). The correlation between *CYP1A1* inducibility and *AhR* levels was not observed in nonsmokers (data not shown).

Since gene expression levels and inducibility differed by gender and smoking status (Table 3), the correlation between gene expression levels was further assessed by multiple linear regressions. The effects of gender and smoking status were controlled in this model. As shown in Table 4, *AhR* levels were positively correlated with *CYP1B1* levels in DMSO and BA-treated cells, but negatively correlated with *CYP1B1* inducibility. *Arnt* levels were also associated with *CYP1B1* levels in BA-treated cells. During data analysis, we observed that cigarette smoking had significant interaction with *AhR* levels (Table 3). The interaction meant that smoking modified the association between *AhR* and *CYP1A1* levels in DMSO-treated cells and *CYP1A1* inducibility. Other interaction terms, such as AhR*gender, Arnt*gender, and Arnt*smoking, were not significant in all models (data not shown). After controlling for gender, smoking, and their interaction, *AhR* levels negatively correlated with *CYP1A1* levels in the DMSO-treated cells, but positively correlated with *CYP1A1* inducibility.

DISCUSSION

CYP1A1 inducibility has been considered as a susceptibility factor for lung cancer and is usually determined by measuring enzyme activity, such as AHH or EROD (Kellermann *et al.*, 1973; Kiyohara *et al.*, 1998). Induction of *CYP1A1* is dependent on AhR activation (Whitlock, 1999). CYP1A1 inducibility was highly variable in human population. Therefore, we hypothesized that *AhR* and *Arnt* expression might count for interindividual variation in CYP1A1 inducibility. Our study showed that gender and cigarette smoking in cultivated lymphocytes affected *CYP1A1* inducibility. We also noticed that cigarette smoking had interaction with *AhR* levels and modified the association between *AhR* levels and *CYP1A1* inducibility. After controlling for gender, cigarette smoking, and their interaction, *CYP1A1* inducibility correlated with *AhR* levels in noninduced lymphocytes. These data partially support our hypothesis that the differences in *AhR* expression, but not in *Arnt* expression, associate with individual variation in *CYP1A1* inducibility.

In DMSO-treated lymphocytes, *CYP1B1* levels were higher than *CYP1A1* levels and positively correlated with *AhR* levels. This was consistent with the results of a previous study, which showed that *CYP1B1* was constitutively expressed in cultivated lymphocytes (Spencer *et al.*, 1999). Shehin *et al.* (2000) have demonstrated that the dioxin response elements in the enhancer region of *CYP1B1* were responsible for the constitutive expression of *CYP1B1*. These data suggest that the AhR signaling pathway regulates the constitutive expression of *CYP1B1* in

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TABLE 4
Associations between *AhR* and *Arnt* and *CYP1A1* and *CYP1B1* Gene Expression Levels and Inducibility

Dependent variable	Independent variable[a]	Parameter estimate (SE)[b]	*p* value for *AhR* or *Arnt*
In DMSO-treated cells			
CYP1A1	*AhR*[c]	–0.55 (0.13)	<0.01
CYP1A1	*Arnt*	–0.04 (0.04)	0.42
CYP1B1	*AhR*	0.21 (0.04)	<0.01
CYP1B1	*Arnt*	0.05 (0.03)	0.08[d]
In BA-treated cells			
CYP1A1	*AhR*	–0.06 (0.06)	0.27
CYP1A1	*Arnt*	–0.01 (0.02)	0.64
CYP1B1	*AhR*	0.16 (0.05)	<0.01
CYP1B1	*Arnt*	0.04 (0.02)	0.01
CYP1A1 inducibility	*AhR* in DMSO-treated cells[c]	0.25 (0.11)	0.02[d]
CYP1A1 inducibility	*Arnt* in DMSO-treated cells	0.02 (0.03)	0.46[d]
CYP1B1 inducibility	*AhR* in DMSO-treated cells	–0.14 (0.05)	<0.01[d]
CYP1B1 inducibility	*Arnt* in DMSO-treated cells	–0.03 (0.03)	0.18[d]

Note. In the multiple linear regression analysis, we controlled for gender and cigarette smoking status.
[a]Other independent variables included in the model were smoking and gender.
[b]Parameter estimate (standard error) in natural logarithm-transformed scale.
[c]Adjusted for smoking and gender, and included the interaction term AhR*smoking in the model; p value for AhR*smoking in this model was <0.05.
[d]p value for gender in this model was <0.05.

lymphocytes. Therefore, in the absence of exogenous AhR ligands, CYP1B1 should play a role in AhR-mediated biological and physiological functions. *CYP1B1* expression was also induced by PAHs in cultivated lymphocytes, although the induction fold of *CYP1B1* was much lower than that of *CYP1A1*. In BA-treated lymphocytes, *CYP1B1* levels positively correlated with *AhR* and *Arnt* levels. However, *AhR* levels negatively correlated with *CYP1B1* inducibility. It is possible that the *AhR*-dependent increase of constitutive *CYP1B1* levels (in DMSO-treated cells) was greater than that of induced *CYP1B1* levels (in BA-treated cells).

Several epidemiological studies have indicated that female smokers are at higher risk of lung cancer than male smokers (Engeland, 1996; Prescott *et al.*, 1998; Zang and Wynder, 1996). Mollerup *et al.* (1999) reported that the levels of hydrophobic DNA adducts in the nontumor lung tissues of female smokers were higher than those of male smokers. Furthermore, *CYP1A1* expression levels correlated with DNA adduct levels in nontumor lung tissues, and were significantly higher in female smokers than in male smokers (Mollerup *et al.*, 1999). Consistent with these results, we observed that *AhR*, *CYP1A1*, and *CYP1B1* levels in noninduced lymphocytes were significantly higher in female nonsmokers than in male nonsmokers. CYP1A1 and CYP1B1 participate in the metabolic activation of PAHs (Shimada *et al.*, 1992, 1996). Many carcinogenic PAHs have been identified in tobacco smoke (Hecht, 1999). These data imply that higher *AhR* expression causes higher *CYP1A1* and *CYP1B1* expression in females.

Cigarette smoking is an important confounding factor for AHH inducibility (Kiyohara and Hirohata, 1997). We found that *CYP1A1* inducibility was significantly higher in male smokers than that in male nonsmokers. Furthermore, the relationship between *AhR*, *CYP1A1* inducibility, and *CYP1A1* in noninduced lymphocytes differed by smoking habit. *AhR* levels positively correlated with *CYP1A1* inducibility in male smokers. However, the correlation coefficient between *AhR* levels and *CYP1A1* inducibility was negative in male nonsmokers, although it was not statistically significant (data not shown). The opposite relationship was also noticed between *AhR* and *CYP1A1* levels in noninduced lymphocytes (data not shown). It is possible that cigarette smoking interfered with the mechanism of *AhR*-mediated *CYP1A1* induction. More experiments are necessary to understand the interaction between cigarette smoking and the AhR signaling pathway.

CYP1A1 genetic polymorphisms were demonstrated to correlate with AHH and EROD inducibility in Japanese populations (Kiyohara *et al.*, 1996, 1998). When we determined the *m1* and *m2* polymorphisms of *CYP1A1* in our samples, these polymorphisms were not associated with *CYP1A1* inducibility in our study (data not shown). This is probably because our sample size was too small.

In the present study, we demonstrated that *AhR* expression correlated with *CYP1A1* inducibility in human lymphocytes and cigarette smoking was an important confounding factor for the relationship. On the other hand, *AhR* expression correlated with *CYP1B*1 expression, but not *CYP1B1* inducibility. In subsequent studies, we will compare *AhR* and *CYP1B1* expression in lung cancer patients and healthy individuals. These studies allow us to understand whether *AhR* and *CYP1B1* expression levels can be used to assess the susceptibility to lung cancer.

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