ABSTRACT:

Cytochrome P450 1B1 is a recently recognized phase I bioactivating enzyme with high affinity for both inhaled tobacco carcinogens and 17β-estradiol. We evaluated the human lung expression of this multifunctional member of the P450 superfamily across 16 individuals. Expression of CYP1B1 was evaluated by qualitative reverse transcription-polymerase chain reaction and Western immunoblots performed on human tumor and nontumor lung tissue. Expression at both mRNA and protein levels was then correlated with smoking history, plasma biomarkers of tobacco exposure (nicotine and cotinine), gender, and tumor histology. CYP1B1 mRNA and protein were detected in 94 and 100% of individuals, respectively. Multivariate analysis confirmed that there were more subjects displaying CYP1B1 mRNA expression in tumor than nontumor tissue (p = 0.0003). Correlation of CYP1B1 protein with plasma cotinine levels was statistically marginal (p = 0.027). Self-reported smoking history, gender, and tumor histology did not correlate with gene expression in the multivariate model. After multivariate modeling for confounding factors, the expression patterns of 5 of 16 individuals appeared to differ from the group as a whole for mRNA and/or protein. We conclude that CYP1B1 is commonly expressed in human lung and hypothesize that it may be an important phase I enzyme with respect to human lung carcinogen metabolism, warranting an understanding of regulatory control and coding region polymorphisms.

Several inherited genetic polymorphisms in carcinogen-metabolizing enzyme genes have been hypothesized as contributing to lung cancer susceptibility. A number of studies have examined carcinogen-metabolizing enzyme activity, such as aryl hydrocarbon hydroxylase activity, as well as several variant cytochrome P450 1A1 (CYP1A1) alleles as they relate to lung cancer. Initial human studies suggested a positive correlation between aryl hydrocarbon hydroxylase inducibility of peripheral blood lymphocytes when stimulated and assayed ex vivo and presence of lung cancer. However, actual CYP1A1 coding sequence polymorphisms, examined in case-control molecular epidemiology studies, have yielded inconsistent results across studies of Euro-American populations (Hirvonen et al., 1992; Shields et al., 1993; Drakoulis et al., 1994; Sugimura et al., 1994; Spivack et al., 1997). Similar inconsistencies apply to the accumulated literature for polymorphisms of CYP2D6 and CYP2E1 genes and their correlation with the presence of lung cancer (Kato et al., 1992; Hirvonen et al., 1993; Agundez et al., 1994; Hamada et al., 1995; Shaw et al., 1995; Bouchardy et al., 1996; Spivack et al., 1997). Therefore, with respect to tobacco-induced lung cancer susceptibility, the identity of the key relevant phase I carcinogen metabolizing genes, and the key polymorphism(s) in those genes, remains unclear.

CYP1B1 bioactivates many of the same exogenous procarcinogens as the well studied CYP1A1, including polycyclic aromatic hydrocarbons, nitro-aromatics, and arylamines (Sutter et al., 1994; Shimada et al., 1996; Kim et al., 1998). While substrate affinity and kinetics differ for the individual xenobiotic for CYP1B1 versus CYP1A1 (Kim et al., 1998), both enzymes are induced by tobacco smoke, and their expression is aromatic hydrocarbon receptor (Ahr)-mediated at one or more xenobiotic response elements (Spink et al., 1998a,b; Eltom et al., 1999). Tobacco-induced expression of CYP1B1 in human lung has been suggested to vary widely interindividually, over several orders of magnitude, as assessed in endobronchial mucosal biopsies taken from active cigarette smokers as compared with nonsmokers (Willey et al., 1997). Thus, variable interindividual expression patterns in human lung are suspected to exist.

There is considerable support in the epidemiologic literature for the position that women are at higher risk than men for lung cancer at any given level of smoking (Ernst, 1994; Ryberg et al., 1994; Zang and Wynder, 1996). The estrogen hormonal environment is thought to synergize with the mutagenicity of inhaled tobacco components. CYP1B1 readily metabolizes 17β-estradiol, with its primary hydroxylase activity at C-4 (Hayes et al., 1996; Spink et al., 1998b). In animal models, 4-hydroxyestradiol is carcinogenic (Liehr et al., 1986; Li and Li, 1987). Mechanisms underlying the interplay of hormonal, genetic, and environmental factors are largely speculative at this time.

In an effort to lay the groundwork for assessments of the role of...
CYP1B1 in lung carcinogenesis, we have assessed CYP1B1 expression at mRNA and protein levels in human lung, used CYP1A1 expression for comparison, and correlated CYP1B1 expression levels with tobacco smoke exposure, gender, lung tissue of origin (tumor versus nontumor), and histologic diagnosis.

Materials and Methods

Subjects. A group of 16 individuals undergoing lung resectional surgery for suspected carcinoma consented to participate in the study and provided lung tissue for analysis. Subjects came from the Albany, NY region, and surgery was performed at either a tertiary care center (Albany Medical Center) or a large community hospital (St. Peter’s Hospital). The study was conducted under the auspices of the respective institutional review boards. Precise mainstream or sidestream tobacco exposure history, self-reported down to the individual were performed using the same RNA isolate from a single subject and stored briefly at room temperature, the plasma fraction was frozen, and volumes were 20.0 μl. CYP1B1 cDNA PCR was accomplished in single stage, 40 cycles, using the following protocol: 95°C for 1-min hotstart; then 40 cycles of 10-s denaturing at 95°C, 15-s annealing at 57°C, then 1-min extension at 72°C; followed by a terminal 7-min extension at 72°C. PCR cycling for CYP1A1 cDNA was performed by nested PCR for maximum sensitivity, using the 1A1GSPF/1AI1G2R primer set in the first stage optimized to 95°C for 1-min hotstart; then 30 cycles of 10-s denaturing at 95°C, 15-s annealing at 55°C, then 1-min extension at 72°C; followed by a terminal 7-min extension at 72°C. Second stage for CYP1A1 cDNA PCR was performed on 1.0 μl of the first stage product under identical hotstart, cycle, and extension conditions using the 1A1JWF/1G1G2R primer set. β-actin cDNA amplification was performed for 30 cycles using identical conditions to that for CYP1B1 cDNA amplification.

Cross reactivities of the primers were checked with GCG-Wisconsin statistical software for sequence analysis (Madison, WI). There was greater than five nucleotide mismatches for 1B1 primers to prime 1A1 transcript or genomic DNA, as was the case for 1A1 primers to prime 1B1 transcript or genomic DNA. None of the sets would prime 1A2 transcript or gene without greater than five mismatches, and this was true for members of the 2C, 2D, 2E, and 3A families of P450s. All runs were performed with positive specific control cDNA (derived from the dioxin-stimulated MCF-7 breast cancer cell line) and water blanks. PCR product was displayed on ethidium bromide gel, photographed under ultraviolet light, and if visually apparent, recorded as “positive”.

Southern blotting of PCR product was performed with a probe annealing specifically to the CYP1B1 PCR product [1B1JZF 5′-CATCGCAACACCTCTGTCTT-3′], which was 5′-end labeled with [32P]ATP using the RTS Kinase Labeling System (Life Technologies, Rockville, MD) and hybridized according to Stratagene’s QuikHyb protocol (La Jolla, CA).

Direct Sequencing of PCR product was performed by first purifying product using a Centri-Sep (Perkin Elmer) column, and then sequenced by the Wadsworth Center’s molecular genetics core facility, using a PerkinElmer Biosystems ABI PRISM 377XL automated DNA sequencer.

Protein Analysis. Microsomal preparation was performed from human lung tissue by a standard technique (Fasco et al., 1993). Briefly, 100 mg of tissue was pulverized in a liquid N2-immersed mortar and pestle and stored briefly at −80°C tissue bank until analyzed. Protein analysis was performed in duplicate.

Protein Analysis. Microsomal preparation was performed from human lung tissue by a standard technique (Fasco et al., 1993). Briefly, 100 mg of tissue was pulverized in a liquid N2-immersed mortar and pestle apparatus and immersed in 1.0 ml of microsomal preparation buffer (0.2 mM phenylmethylsulfonyl fluoride, 1.0 mM dithiothreitol, 1 mM EDTA, 20 mM Tris acetate, 0.14 M KCl). Samples were then sonicated for 15 s and centrifuged at 12,000g for 20 min at 4°C. The supernatant was ultracentrifuged at 100,000g for 60 min at 4°C, and the pellet was resuspended in 0.5 ml of microsomal storage buffer (50 mM Tris acetate, 1.0 mM EDTA, 20% glycerol, 0.2 mM phenylmethylsulfonyl fluoride, 1.0 mM dithiothreitol). Microsomal protein was quantified by BCA Protein Assay kit (Pierce, Rockford, IL). Multiple replicate protein expression trials for an individual were performed on the same microsomal isolation from a single sample.

Western immunoblotting was performed in a Bio-Rad assembly (Hercules, CA) using standard running buffer (25 mM Tris HCl, 0.192 M glycine, 0.1% sodium dodecyl sulfate) and transfer buffer (25 mM Tris HCl, 0.192 M glycine, 0.01% sodium dodecyl sulfate, 20% methanol). Each lane was loaded with 5 μg of microsomal protein, with positive control lanes containing 2.4, 10.2, or 51.0 fmol of lymphoblast-expressed CYP1B1 or 1A1 protein (GENTEST, Woburn, MA), and the gel was run at 100 V for 1.5 to 2.5 h for maximum resolution. Blotting was performed with 5% nonfat dry-milk, 0.25% Tween 20, 1% bovine serum albumin, and 5% goat (CYP1B1) or rabbit (CYP1A1) serum in phosphate-buffered saline solution for 2 h at 23°C and then in 5% nonfat dry-milk, 0.25% Tween 20 overnight. Primary rabbit anti-human CYP1B1 peptide IgG was kindly provided by Dr. F. Kadlubar (NCTR, Little Rock, AR; Tang et al., 1999). Anti-human CYP1A1 goat IgG was purchased from GENTEST. Both anti-CYP1B1 and 1A1 antibody titers were optimized at 1:400, applied in blocking solution for 2 h at 23°C, washed in phosphate-buffered saline-Tween 20, and followed with an anti-IgG antibody, appropriate to rabbit or goat primary IgG, respectively. The secondary antibodies were purchased pretagged with horseradish peroxidase (Sigma, St. Louis, MO) and applied in previously optimized titers of 1:4,000 (for CYP1B1) or 1:5,000.
(for CYP1A1). Identical substrates were used in each assay for chemiluminescent detection (Pierce Supersignal, Rockford, IL) per the manufacturer’s protocol. Kodak X-OmatAR film was exposed from 10 s to 2 h, depending on signal strength, and visible target bands categorized as positive.

**Nicotine and Cotinine Analysis.** Plasma nicotine and cotinine levels were measured using a modification of a standard procedure (Davis, 1986). Five hundred microliters of plasma spiked at 100 ng/ml with deuterated nicotine and cotinine internal standards was diluted with 500 μl of 5 M sodium hydroxide and extracted with methylene chloride. The organic layer was concentrated to dryness, and the solvent was changed to 2-propanol and reduced to 10 μl. Gas chromatography/mass spectrometry was used to separate and detect nicotine and cotinine using the deuterated internal standards to quantitate the amount of nicotine and cotinine present in plasma. The limit of detection for nicotine was 5.0 ng/g of plasma, and that for cotinine was 10.0 ng/g of plasma.

**Data Analysis.** The univariate analyses explored the factors, taken one at a time, that correlated with a subject ever having been positive for expression of either of the two genes among multiple replicate trials (Table 1, Fig. 2). This ever-positive criteria, where one positive among multiple replicate experimental trials is sufficient to label the subject as positive for that gene’s expression at the mRNA or protein level, is standard analysis. Fisher’s exact test (Agresti, 1992) was used to evaluate the fraction of the 16 subjects “ever-positive” for one measure of expression (e.g., 1B1 mRNA tumor tissue) versus the fraction ever-positive for another marker of expression (e.g., 1B1 mRNA nontumor tissue). p values express the probability that, for the given sample sizes, the observed difference between the compared fractions ever-positive was a random occurrence. Bonferroni adjustment (Seber, 1997) for multiple comparisons was used in this analysis to establish conservative significance levels at 0.05/n, where n = number of comparisons made in the particular analysis.

For the multiple logistic analysis, each replicate experiment was modeled as a function of multiple indicators for the individual patient, assay date, tissue source (tumor/nontumor), gender, smoking history (current mainstream active; recent mainstream, within two weeks; current environmental tobacco smoke; former smoker; never smoker), nicotine/cotinine concentration (with 0 as below detection), histologic diagnosis. For each measurement, binary regression parameters were estimated by likelihood maximization (Cox, 1970). After fitting full models, single variable backward elimination was used to select a model containing only significant effects as judged by the chi-squared likelihood ratio test statistic (Bishop et al., 1975). Bonferroni adjustment for multiple comparisons was used in this analysis.

**Results**

For our 16 subjects, self-described tobacco exposure was distributed as follows: light exposure (n = 2), former smokers (n = 7), recent smokers (within 1–2 weeks, n = 2), and current mainstream smokers (as of preoperative interview, n = 5). There was no detectable nicotine present in any of the plasma samples, implying tobacco exposure preceded the collection of blood at interview by at least several hours and by inference the harvest of lung tissue by several days. There were measurable cotinine levels in two of the four current mainstream smokers, consistent with this inference. The group was comprised of 4 females and 12 males and included an assortment of lung cancer and noncancer histologic diagnoses (Table 1).

The frequency of CYP1B1 expression at mRNA and immunoreactive protein levels is summarized in Table 1 and Figs. 2 and 3, and representative gels are displayed in Fig. 1. A and B. CYP1A1 expression was assayed for reference. The presence of CYP1B1 message was confirmed by both Southern blotting of the PCR product and by direct PCR product sequencing of a subset of samples that excluded

### TABLE 1

Subject characteristics, exposure history, and detection frequency of CYP1B1 expression

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Smoking History</th>
<th>Plasma Cotinine</th>
<th>Histologic Diagnosis</th>
<th>CYP1B1 mRNA*</th>
<th>CYP1B1 Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>Current MS, light</td>
<td>N.D.</td>
<td>Adenocarcinoma</td>
<td>pos (6/6)</td>
<td>pos (6/7)</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>Former MS</td>
<td>N.D.</td>
<td>Squamous cell carcinoma</td>
<td>pos (4/4)</td>
<td>pos (4/4)</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>Current ETS</td>
<td>N.D.</td>
<td>Squamous cell carcinoma</td>
<td>pos (4/4)</td>
<td>pos (3/3)</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>Former MS</td>
<td>N.D.</td>
<td>Adenocarcinoma</td>
<td>pos (1/3)</td>
<td>pos (2/3)</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>Recent MS</td>
<td>112</td>
<td>Tuberculosis</td>
<td>pos (9/9)</td>
<td>pos (2/3)</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>Recent MS</td>
<td>54</td>
<td>Adenocarcinoma</td>
<td>pos (6/6)</td>
<td>pos (2/5)</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>Recent MS</td>
<td>N.D.</td>
<td>Squamous cell carcinoma</td>
<td>pos (2/9)</td>
<td>pos (2/2)</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>Former MS</td>
<td>N.D.</td>
<td>Bronchialveolar cell carcinoma</td>
<td>pos (1/3)</td>
<td>pos (5/5)</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>Former MS</td>
<td>N.D.</td>
<td>Bronchialveolar/adenocarcinoma</td>
<td>pos (2/4)</td>
<td>pos (6/6)</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>Current MS</td>
<td>176</td>
<td>Adenocarcinoma</td>
<td>pos (2/9)</td>
<td>pos (2/2)</td>
</tr>
<tr>
<td>11</td>
<td>Male</td>
<td>Former MS</td>
<td>N.D.</td>
<td>Squamous cell carcinoma</td>
<td>neg (0/4)</td>
<td>neg (0/2)</td>
</tr>
<tr>
<td>12</td>
<td>Female</td>
<td>Former MS</td>
<td>N.D.</td>
<td>Non-small cell carcinoma</td>
<td>pos (5/5)</td>
<td>pos (6/7)</td>
</tr>
<tr>
<td>13</td>
<td>Male</td>
<td>Current MS (pipe)</td>
<td>N/A</td>
<td>Non-small cell carcinoma</td>
<td>pos (3/7)</td>
<td>pos (8/10)</td>
</tr>
<tr>
<td>14</td>
<td>Male</td>
<td>Former MS</td>
<td>N.D.</td>
<td>Metastasized to lung (clear cell)</td>
<td>pos (1/3)</td>
<td>pos (4/7)</td>
</tr>
<tr>
<td>15</td>
<td>Female</td>
<td>Current MS, light</td>
<td>292</td>
<td>Non-small cell carcinoma</td>
<td>pos (6/7)</td>
<td>pos (7/8)</td>
</tr>
<tr>
<td>16</td>
<td>Male</td>
<td>Former MS</td>
<td>N/A</td>
<td>Metastasized to lung (clear cell)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

MS, mainstream tobacco smoke; ETS, environmental (sidestream) tobacco smoke; N/A, tissue unavailable.

* In expression columns, pos = any replicate experiment positive; neg = no replicate trials positive.
any detectable CYP1A1 in the PCR product. All mRNA and protein experiments were run with appropriate positive and negative controls for both tissue quality and assay integrity.

Data were analyzed using two approaches. First, the univariate analyses examined factors one-at-a-time and correlated these individual factors with gene expression. In this analysis, gene expression was determined by traditional “ever-positive on replicate experiments is positive” criteria to categorize subjects as expressors or nonexpressors. CYP1B1 mRNA and protein were consistently expressed across 75% of subjects for both tumor and nontumor tissue (Fig. 2). Significantly more subjects expressed CYP1B1 than CYP1A1 mRNA and protein; this was true in both tumor and nontumor tissue \( (p < 0.0019) \). The CYP1B1 mRNA and protein expression did not differ between tumor and nontumor tissue \( (p > 0.05) \) by this univariate analysis.

The second approach analyzed multiple factors that were associated with gene expression. In this analysis, the fraction of replicate experiments that showed positive gene expression was modeled as the dependent variable. Plausible clinical or biological factors that might impact on the expression of CYP1B1 were modeled against the fraction of trials that were positive. There was substantial variability between individuals in the expression of CYP1B1 message and protein (Fig. 3), not easily explained by multivariate modeling for confounders (Table 2). For example, pooling expression data from both tumor and nontumor tissue, subjects 2 and 5 had a higher fraction of replicate trials demonstrating CYP1B1 mRNA; subject 11 appeared to have a lower fraction of replicate trials demonstrating CYP1B1 mRNA than the group as a whole. For CYP1B1 protein, subjects 2 and 8 demonstrated a higher fraction of replicate trials that were positive, whereas subject 6 was lower than the group as a whole. Discordant mRNA and protein results for several individuals were notable and suggested differing kinetics for the two measures of gene expression. Another predictive factor explaining some of the CYP1B1 mRNA expression frequency variance was tissue source; CYP1B1 mRNA expression frequency in tumor tissues exceeded that demonstrated in nontumor tissue \( (p = 0.0003) \). When integrating Bonferroni multiple comparisons corrections for statistical significance \( (p = 0.002) \), CYP1B1 protein expression was not higher in the tumor \( (p = 0.03) \). Similarly, plasma cotinine levels did not linearly increase with CYP1B1 protein \( (p = 0.027) \). Self-described smoking status displayed no correlation with these measures of P450 expression. Gender and tumor histology did not correlate with CYP1B1 expression in this group of subjects.

**Discussion**

We have demonstrated the expression of the carcinogen- and estrogen-bioactivating enzyme CYP1B1 in human lung tissue, across individual subjects, at the mRNA and protein levels. We have, to the best precision available in an observational study, defined the tobacco exposure of the individuals, historically and by plasma biomarkers, and related that to gene expression levels. We have analyzed CYP1B1 expression frequency at mRNA and protein levels, using the common “ever-positive is a positive” standard, by traditional univariate anal-
and gene expression. As part of this secondary, multivariate analysis, detailed statistical approach to correlate biological and clinical factors univariate primary analysis for our qualitative data, we applied a more subject replicates positive. Thus, in addition to the traditional replicates positive versus another subject with two of nine (e.g., using an ever-positive criteria does not make use of the expression quantitative analysis by conventional techniques. Univariate analysis replicates experiments. analyses and also used multivariate techniques in the analysis of our replicate experiments.

The occurrence of CYP1B1 and protein at the threshold of detectability in these human specimens makes it impractical to perform quantitative analysis by conventional techniques. Univariate analysis using an ever-positive criteria does not make use of the expression difference between a subject with nine of nine (e.g., subject 5) replicates positive versus another subject with two of nine (e.g., subject 7) replicates positive. Thus, in addition to the traditional univariate primary analysis for our qualitative data, we applied a more detailed statistical approach to correlate biological and clinical factors and gene expression. As part of this secondary, multivariate analysis, we counted each replicate experimental trial on a sample as an observation, analyte presence or absence. This approach avoids arbitrary cutoffs on what fraction of trials is an appropriate threshold to summarize all of them as concordantly positive or “negative”, allows examination of individual experimental trial dates as possible confounders, and increases the power of the multivariate analyses without losing data in such trial-set summaries as positive or negative. Thus, in Table 1, the four appropriately controlled trials of CYP1B1 RT-PCR for the tumor tissue from subject 9 were counted as two trials positive and two negative, a total of four observations, rather than applying an arbitrary judgment as all positive or negative. Working at the limits of sensitivity for the respective assays, where trial-to-trial variability is observable, despite proper positive and negative controls performing their respective functions with each trial adequately, we believe this was a conservative and rigorous way to analyze the data. Most of our data on any given individual were concordant among that individual’s trials. The numbers of replicate trials performed for any individual differed were based on several factors: a) tissue availability; b) inclusion of only valid replicate experiments, judged by adequacy of positive and negative controls; c) concordance of results of previous trials; and d) historical smoking status. The multiple replication of trials, to a maximum of 10 in those with provocative “current” or “recent” cigarette smoke exposure did not confound results, as there was no relationship between historical smoking status and any of the biomarkers of gene expression studied.

Our raw data, and univariate and multivariate results, differ from those of Hakkola et al. (1997), who reported no measurable CYP1B1 mRNA in a small number of “pooled” human postsurgical lung samples. That same group did detect CYP1B1 mRNA in alveolar macrophages lavaged from volunteers, as well as in nonlung organs. RNA degradation is always suspect when tissues yield negative results. We assume the pooling of different subjects’ tissues in that study did not confound the analysis and that their negative results are not due to individual trial data summaries that reduce experimental power, as discussed above. We speculate that their use of a DNase technique to exclude contaminating genomic DNA from the RNA isolation and subsequent cDNA amplification in their study may be problematic. The use of an RNase-free DNase treatment regimen to address genomic DNA contamination of RNA extracts can result in dose-dependent degradation of RNA (Huang et al., 1996), despite strict precautions. Certainty of expression for human lung CYP1B1 mRNA by RT-PCR in our study was conferred by a) the dichotomous nature of a run (present or absent); b) the design of the CYP1B1 primers to span the intron between exons 2 and 3 that was kinetically unfavorable and visually recognizable for contaminating genomic DNA PCR (product length 3716 bp) compared with the cDNA PCR product (684 bp); c) the agreement of the CYP1B1-DS primer set results with a second designed primer set; d) product size from any possible CYP1A1 cDNA product not observed and discordant from the CYP1B1 cDNA product size from this primer set; e) the trial repetition of three to nine times per tumor or non tumor sample; and f) in a subset of samples from several subjects, confirmation by both Southern blotting and direct sequencing of the PCR product.

The presence of human lung CYP1B1 protein identified by immuno blot in our series of patients is in agreement with other, smaller studies (Murray et al., 1997; Tang et al., 1999). Certainty of protein expression for our study lies in the specificity of the anti-peptide primary antibody used (Tang et al., 1999), as well as in our own current data (Fig. 1B). A primary antibody omission study confirmed this specificity; an absence of primary CYP1B1 antibody extinguished the CYP1B1 signal in both experimental and control lanes.

The explanation for interindividual heterogeneity of CYP1B1 ex-

**TABLE 2**

Multiple logistic regression of CYP1B1 expression using fraction of experimental replicate trials positive as the dependent variable.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>CYP1B1 mRNA</th>
<th>CYP1B1 Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor tissue (vs. nontumor tissue)</td>
<td>0.0003</td>
<td>0.0312</td>
</tr>
<tr>
<td>Cotinine level (plasma)</td>
<td>0.0271</td>
<td>---</td>
</tr>
<tr>
<td>Smoking history (self-reported)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Gender</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Tumor histology</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

---

Individual subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>CYP1B1 mRNA</th>
<th>CYP1B1 Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>&lt;0.0001</td>
<td>0.0423</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>---</td>
<td>0.0015</td>
</tr>
<tr>
<td>8</td>
<td>0.0191</td>
<td>0.0213</td>
</tr>
<tr>
<td>11</td>
<td>0.0056</td>
<td>---</td>
</tr>
<tr>
<td>12</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
</tbody>
</table>

---

*---, p > 0.05 (nonsignificant).*
pression is not mechanistically addressed by this study. The regulatory control of members of the cytochrome P450 superfamily is complex, varying across individual gene and cell type. Control of CYP1B1 and CYP1A1 expression is thought to involve the binding of a polycyclic aromatic hydrocarbon ligand to Ahr, binding of hsp-90 to the Ahr-ligand complex and translocation to the nucleus, heterodimerization with Arnt, release of hsp90, and interaction with enhancer regions within the 5′ regulatory region (Whitlock et al., 1996; Rowlands and Gustafsson, 1997). One can therefore envision a variety of sites and mechanisms resulting in polymorphic CYP1B1 transcription, and by extension, translation. There are probably non-Ahr-dependent factors, some of them cell-type specific, regulating expression of these genes as well (Hakkola et al., 1997; Spink et al., 1998b). Thus, our homogenized lung tissues contain epithelial and nonepithelial cell types, the precise ratio uncontrolled across samples containing identical quantities of RNA or protein, and thus varying across individuals, possibly spuriously introducing heterogeneity of P450 expression (Christou et al., 1995).

Frequency of expression of CYP1B1 at message (p = 0.0003) and possibly protein (p = 0.03) levels was statistically greater in tumors than non-tumors, by the multivariate analysis. This finding is consistent with an immunostaining study in a broad range of tissues (Murray et al., 1997) and in breast tumors (McFayden et al., 1999). We did not compare the expression of CYP1B1 in the noninvolved lung tissue of those with lung cancer versus those without lung cancer (there was only one patient without a chest malignancy in this series). Therefore the significance of “underexpression” of CYP1B1 in non tumor tissue with respect to carcinogenesis is not clear. However, this finding of elevated tumor CYP1B1 expression could potentially be used therapeutically for preferential bioactivation of anticancer prodrugs.

Several factors did not contribute to expression at the mRNA level of CYP1B1 including self-reported smoking history. Self-reported smoking history was assessed several days before surgery, during the preoperative interview and phlebotomy session, thus probably unlinking smoking history from tissue gene expression. Furthermore, given identical cigarette pack-year exposure histories, there is wide variation in the literature on resulting CYP1A1 expression, and we infer the same is true for CYP1B1, largely because proximate tobacco exposure is probably more relevant than total lifetime tobacco exposure for P450 expression measurements. A previous study by Willey et al. (1997), using bronchial mucosa cells and biopsies from healthy current smokers and nonsmokers, suggested induction of these genes’ expression was generally detectable by quantitative PCR, although there was considerable individual variation across several orders of magnitude. We confirmed smoking status by plasma nicotine and cotinine levels. Plasma cotinine levels in our subjects was weakly associated in the multivariate model with protein level expression of CYP1B1 or CYP1A1, its turnover kinetics may parallel those of the respective carcinogen-metabolizing enzyme induced by other components of the tobacco smoke mixture. Neither gender nor tumor histology correlated with the expression pattern for CYP1B1 or CYP1A1 by either univariate or multivariate analyses within the statistical power of the study.

We conclude that CYP1B1 is commonly expressed in human lung at both mRNA and protein levels. Given that previous studies suggest this enzyme has carcinogen-metabolizing activity for many tobacco-smoke constituents, and has substantial 17β-estradiol metabolism capacity with resultant carcinogenicity, one may speculate on a link between these respective metabolic roles and the human epidemic of lung cancer among females. Further study of the regulatory control of this gene is under way in this and other laboratories.

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**References**


