ABSTRACT

Cytochrome P450 (CYP) enzymes expressed in human lung can metabolize a variety of xenobiotics, drugs, and endogenous compounds. Metabolism of these substrates may lead to their detoxification or activation and may affect the homeostasis of the lung, its susceptibility to disease, response to therapy, and clinical prognosis. We analyzed the expression of CYP2B7, CYP4B1, and NADPH-cytochrome P450 oxidoreductase (OR) mRNAs in normal lung controls, normal lung from lung cancer patients, and lung tumors using the sensitive technique of RNase protection. The mRNAs of CYP2B7, CYP4B1, and OR were detected in all the normal and a majority of neoplastic tissues. The three mRNAs were quantified and found at an average ratio of 0.89, 4.03, and 0.88% relative to actin mRNA in normal lung, respectively. There was no correlation between the levels of expression of the three mRNAs and the histological diagnosis of tumors. The amounts of each of the three mRNAs varied considerably between patients, but analysis of frequency distribution of the levels of CYP2B7 and CYP4B1 mRNAs did not present evidence for genetic polymorphism as a possible source of the observed interindividual variability. Levels of expression of the two P450 mRNAs were reduced (2.3- and 2.4-fold) in the neoplasms compared to normal lung. The level of OR mRNA expression was uniform with no significant differences between normal and neoplastic tissues, and its interindividual variability was the lowest amongst the three mRNAs studied. All mRNAs had increased interindividual variability in neoplastic tissues. Analysis of the patients' smoking histories and the level of CYP2B7, CYP4B1, and OR mRNAs revealed no evidence for their induction by compounds present in cigarette smoke. This study identifies and characterizes lung and lung tumor mRNAs encoding enzymes that may participate in the metabolism of xenobiotics in humans.

INTRODUCTION

CYPs are heme-containing enzymes capable of oxidizing a variety of xenobiotics such as environmental pollutants, chemical carcinogens, and drugs as well as selected endogenous substrates (1–3). Metabolism of known procarcinogens by P450 proteins may lead to their detoxification or to the formation of reactive metabolites. Interactions of reactive metabolite with cellular macromolecules may lead to cellular toxicity and carcinogenicity (4).

Several P450 genes including 1A1, 2F1, and 4B1 are expressed in human lung, and some of them may be specific for that organ (5–7). They can affect the toxicity of environmental airborne pollutants, cigarette smoke, or circulating lung-specific poisons (8). Although the exposure to noxious toxins and cigarette smoke is associated with lung disease including induction of lung tumors, the expression of P450 genes in normal or neoplastic human lung has not been studied in detail. The expression of CYP1A1 mRNA has been characterized in the extensive collection of tumors and adjacent normal lung by Northern hybridization technique (9). This P450 is highly inducible by tobacco smoke and is present at low levels in nonsmoking subjects.

Quantification of CYP2B7, CYP4B1, and CYPOR Messenger RNAs in Normal Human Lung and Lung Tumors

Maciej Czerwinski, Theodore L. McLemore, Harry V. Gelboin, and Frank J. Gonzalez

Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20892 [M. C., H. V. G., F. J. G.], and Pulmonary Division, St. Joseph’s Hospital, Paris, Texas 75460 [T. L. M.]

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1 To whom requests for reprints should be addressed, at Department of Health and Human Services, NIH, National Cancer Institute, Bldg. 37, Room 3E24, Bethesda, MD 20892.

2 The abbreviations used are: CYP, cytochrome P-450s; OR, NADPH-cytochrome P-450 oxidoreductase; cDNA, complementary DNA.

Other P450s are constitutively expressed in pulmonary tissue. In humans, CYP2B7 and CYP4B1 dominate (6, 10). Currently there is no information on their inducibility or substrate specificity. In the present study, we determined expression of CYP2B7 and CYP4B1 mRNAs in normal and cancerous human lung tissues. We also evaluated mRNA levels for OR, a flavoprotein responsible for transferring electrons from NADPH to the different forms of cytochrome P450 (11).

The total amount of P450s in human lung is significantly lower than in liver. The detection of specific gene products with antibodies against homologous P450 from rat is inherently difficult (12). Therefore, we employed the sensitive technique of RNase protection that can quantitate small amounts of RNA and has been recently applied to the study of CYP2B genes in rat (13) and cytochrome P450 reductase in human tissues (14).

In the present report, tissue-specific expression of CYP2B7 in human lung and CYP2B6 in human liver are described. Levels of CYP2B7, CYP4B1, and OR mRNAs were evaluated in a number of normal and neoplastic lung specimens. The relationship between the level of expression of the three mRNAs and cigarette smoking was also explored.

MATERIALS AND METHODS

The neoplastic and normal pulmonary tissues used in this study were collected at the time of thoracotomy of lung cancer patients at the Johns Hopkins University School of Medicine (9). Histological diagnosis of the neoplasms and patients’ smoking histories were collected and are presented in Table 1. The lung tissues from nonlung cancer patients were collected from kidney donors and supplied to us by Dr. Allan Rettie, University of Washington, Seattle. The source of 10 human livers used in this study has been published (10).

Total RNAs were extracted using cesium chloride gradient centrifugation and were checked for integrity by staining with ethidium bromide following electrophoretic separation in agarose gels containing formaldehyde (15). Only undergraded samples were used.

A papillary adenocarcinoma cell line H441 was obtained from the American Type Cell Collection (Rockville, MD) and cultured as recommended. Total RNA was extracted from confluent cultures as described (16).

The RNase protection assay was performed essentially as described (17, 18). A 5′–3′-850-base pair EcoRI fragment of the cDNAs for CYP2B6 (2B6) and CYP2B7 (2B7) was cloned into pGEM-TZ (Promega Corp., Madison, WI). The plasmid containing CYP2B6 or CYP2B7 cDNA fragment was digested with FokI or DdeI endonucleases, respectively. The antisense RNA probes specific for either mRNA were synthesized using T7 and SP6 RNA polymerases purchased from Promega Corp. The sizes of the protected fragments were 437 base pairs for 2B6 and 395 base pairs for 2B7 mRNAs. The specificity of the antisense RNA probes for either mRNA was confirmed using T7 and SP6 RNA polymerases. The plasmid was digested with FokI or DdeI endonucleases, respectively. The antisense RNA probe specific for either mRNA was synthesized using T7 and SP6 RNA polymerases purchased from Promega Corp. The sizes of the protected fragments were 437 base pairs for 2B6 and 395 base pairs for 2B7 mRNAs. The specificity of the probes was confirmed using 17 single base pair mismatches between CYP2B6 and CYP2B7 cDNAs located in a 400-base pair portion of the coding region of the mRNAs. A 2146-base pair EcoRI fragment of CYP4B1 cDNA was cloned into plasmid pBluescript II KS+ (Stratagene, La Jolla, CA). The plasmid was digested with Xbal restriction endonuclease, and T3 RNA polymerase was used to generate the antisense probe. The size of the mRNA fragment protected by this probe was 380 base pairs. For generation of the oxireductase antisense RNA probe, a 2398-base pair EcoRI fragment of cDNA was inserted into pGEM-3Z in 5′–3′ orientation. The plasmid was digested with Ncol restriction endonuclease, and the probe was synthesized using SP6 RNA polymerase to avoid long stretches of AT located at the 3′ end of the cDNA. The size of the mRNA fragment protected by this probe was 597 base pairs. The fragment of mRNA
Table 1: Histology and smoking status of lung cancer patients

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>15</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>16</td>
</tr>
<tr>
<td>Small cell</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
</tbody>
</table>

Smoking status

- Current smoker: 15, 11, 14, 11, 8, 6
- Non-smoker*: 15, 11, 14, 11, 8, 6
- Smoker: 29, 17, 27, 13, 15, 8

* Have not smoked for more than 6 months prior to the tissue resection.

RESULTS

CYP2B6 and CYP2B7 mRNAs in Normal Lung and Liver and Lung Tumors. In the RNase protection assay, a labeled RNA probe hybridizes to cellular RNA and protects it against digestion with single-strand specific RNases. The application of this sensitive technique allowed us to detect and distinguish between 2B6 and 2B7 mRNAs that are 95% identical across their nucleotide sequence. Typical autoradiographs illustrate the 2B6, 2B7, and the 4B1 mRNAs in lung and liver as well as the variable levels of expression of 2B7 in normal lung from lung cancer patients (Fig. 2, A and B). The actin probe yielded two protected bands of closely related transcripts which were quantified together. The 2B7 mRNA was detected in all normal lungs tested and in 27 of 28 lung tumors. This mRNA was found in liver tissues and, as noted in an earlier study using Northern blot analysis (10), a large degree of interindividual variability was apparent (Fig. 3). In contrast, the 2B6 mRNA was not detected in the normal lungs or lung neoplasms examined. The average 2B7 mRNA level in normal lung from lung cancer patients was 0.89% (SD = 0.74; n = 44) relative to actin mRNA and was significantly lower (0.38%; SD = 0.26; n = 2) than in the neonlastic lung tissue (Wilcoxon rank sum test, P < 0.05). Levels of the three mRNAs in the normal lung from patients of different diagnoses were analyzed together because no significant correlation between pathological classification of tumors and the levels of mRNAs was found. Also, the levels of the three mRNAs in the tumors of different histology did not differ significantly (Wilcoxon rank sum test, P > 0.05). The amount of 2B7 mRNA in normal lung averaged 2.3-fold higher than in the tumor tissues. The interindividual variation in the level of expression of 2B7 gene in normal lung was 14-fold, whereas in the tumors it reached 30-fold.

Fig. 4, A and B shows the distribution of the amount of 2B7 in normal lung from lung cancer patients and in the neoplasms. The levels of 2B7 were also analyzed in six samples of normal lung obtained from kidney donors. The mean value for that group was 1.05% (SD = 0.62), and it was not statistically different from normal lungs from lung cancer patients.

CYP2B7 mRNA in Lung Tumor Cell Lines. In order to evaluate expression of 2B7, 4B1, and OR mRNAs in cell culture models of human lung cancers, we evaluated certain lung tumor cell lines used in drug screening (20). The RNase protection assays to detect the expression of 2B7 were performed on total RNAs from the following 16 lung tumor cell lines: adenocarcinomas (A549, Calu-6, EKVX, H23, H441, and HOP 62); squamous cell carcinomas (H125, H226, H520, and SK-MES-1); large cell undifferentiated carcinomas (A247 and H460); small cell carcinomas (DMS114, DMS273, and H146); and bronchiolo-alveolar carcinoma (H322). The autoradiographs of this experiment did not reveal any 2B6 mRNA-protected band. The 2B7 mRNA was detected in only one cell line, H441. Interestingly, in these cells, the amount of the 2B7 message was relatively high, 3.9% of actin message, which was about 4-fold the amount found in normal lung and 10-fold the average of the tumors examined.

CYP4B1 mRNA in Normal Lung and Lung Tumors. All samples of normal lung from lung cancer patients revealed the band corresponding to the 4B1 mRNA when examined by the RNase protection assay. This mRNA was also detected in 21 of 24 tumors analyzed. The autoradiograph in Fig. 2B illustrates a typical experiment. The amount of 4B1 mRNA quantified in relation to actin was, on average, 4.03% (SD = 2.08; n = 41) in normal lung from lung cancer patients and 1.69% (SD = 1.72; n = 41) of actin mRNA in tumors constituting a statistically significant 2.3-fold reduction of expression of this mRNA in the neoplasms (Wilcoxon rank sum test, P < 0.05). Interindividual variation of the 4B1 mRNA among the patients was 33-fold in normal lungs, whereas in tumors it was as much as 64-fold between the highest and the lowest detectable levels. The distribution of 4B1 mRNA values recorded for individual samples of normal lung and tumors are presented in Fig. 4, C and D. The values for specific histological groups are in Table 2. The six samples of normal lung from kidney donors had the level of 4B1 mRNA of 1.51% (SD = 1.29) of actin mRNA. We attribute the difference between this group and the normal lung from lung cancer patients to its small sample size and do not assign it a biological significance.
NADPH-Cytochrome P450 Oxidoreductase mRNA in Normal Lung and Lung Tumors. The OR mRNA was detected and quantitated in all normal lung from lung cancer patients and tumor specimen examined. Fig. 2C shows the result of a typical experiment detecting OR mRNA. Liver RNA is a positive control in this experiment. The OR mRNA averaged 0.88% (SD = 0.32; n = 23) of actin mRNA in normal lung and 0.65% (SD = 0.25; n = 14) of actin mRNA in the neoplasms. The reduction of the level of expression of OR gene in the neoplasms as compared to normal lung was less pronounced than that of the CYP genes and reached only 1.4-fold. This reduction in the expression was not statistically significant when tumors and normal lungs grouped by histological diagnosis were compared by Wilcoxon rank sum test (P > 0.05). The interindividual variation of the level of the OR mRNA was the lowest of the three mRNAs investigated in this study, 5.3-fold in normal lung and 6.9-fold in the lung tumors. These values are compared with values for other groups in Table 3. The distributions of amount of the OR mRNA in normal lung and tumors resemble that of normal distribution seen with CYP genes and are illustrated in Fig. 4, E and F. The mean value of oxidoreductase mRNA in six normal lungs from kidney donors was 0.98% (SD = 0.24) of actin mRNA and was not significantly different than that of normal lung from lung cancer patients.

The Effect of Smoking on the Levels of CYP2B7, CYP4B1, and OR mRNAs in Normal Lung and Lung Tumors. When the level of CYP1A1 mRNA was evaluated in the collection of normal lung from lung cancer patients and lung tumors used in our study, it was found to be increased by cigarette smoking (9). Using smoking history data, specifically the time of cessation of smoking prior to the thoracotomy, we investigated the possibility of the induction of 2B7, 4B1, and OR mRNAs by compounds present in cigarette smoke. The levels of the three mRNAs were plotted against the time of cessation of smoking prior to the tissue resection. Linear regression analysis was performed and is illustrated in Fig. 5. The three correlation coefficients ($r^2$) were below 0.01, indicating that there is no induction of any of these mRNAs by cigarette smoking.

DISCUSSION

We quantified the level of mRNA expression of two P450 genes, CYP2B7 and CYP4B1, and NADPH-P450 oxidoreductase in normal lung and lung tumors by sensitive RNase protection technique. P450s participate in metabolism of environmental pollutants, procarcinogens, and chemotherapeutic agents, and their metabolic activity is supported by NADPH-cytochrome P450 oxidoreductase. P450 enzymes participate in initiation of tumors by activating procarcinogens to their active forms (2). Therefore, the documentation of the level of expression of these genes in normal tissues and neoplasms of various histological types contributes to an understanding of their potential role in homeostasis and pathology of human lung. It may also point out differences between individual’s normal and neoplastic tissues that can be exploited in disease prevention and treatment.

Normal Lung. The 2B7, 4B1, and OR mRNAs were detected in all normal tissues from lung cancer patients as well as in the small group of normal lung from kidney donors. Since OR is necessary for...
P450 metabolic activity, our demonstration of all three mRNAs documents existence of a multi-function oxidase system in neoplastic and normal lung. Several P450s are present in human lung (12), and one has been purified and identified as CYP1A1 (7). This report is the first quantification of the P450 and OR mRNAs in a large group of normal and neoplastic human lung.

The absence or low levels of the 2B6 mRNA in lung and the analysis of 2B7 mRNA in a group of normal livers evidence for tissue specific expression of the 2B7 gene in human lung. (The CYP2B7 mRNA was also absent in normal kidney and thyroid RNAs.) The distinction between highly similar 2B6 and 2B7 mRNAs would not be possible using Northern blotting. It illustrates an advantage offered by RNase protection assay in analysis of closely related species of mRNA. The 2B7 cDNA contains an internal, in-frame, TGA stop codon (10). The mechanism of translation of this mRNA is currently under investigation in our laboratory. The specificity of expression of

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3. M. Czerwinski, unpublished observations.
Table 2 Levels of CYP2B7, CYP4B1, and OR mRNAs in normal lung from lung cancer patients and lung tumors grouped according to histological diagnosis

<table>
<thead>
<tr>
<th>Histology</th>
<th>CYP2B7 Normal</th>
<th>CYP2B7 Tumor</th>
<th>CYP4B1 Normal</th>
<th>CYP4B1 Tumor</th>
<th>OR Normal</th>
<th>OR Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>All types</td>
<td>0.89 (0.74)*</td>
<td>0.39 (0.26)</td>
<td>4.03 (2.08)</td>
<td>1.69 (1.72)</td>
<td>0.88 (0.32)</td>
<td>0.65 (0.25)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1.04 b</td>
<td>0.43 b c</td>
<td>4.002 (1.94)</td>
<td>1.31 b c</td>
<td>0.81 b</td>
<td>0.78 b</td>
</tr>
<tr>
<td></td>
<td>(0.77)</td>
<td>(0.26)</td>
<td>(1.02)</td>
<td>(0.35)</td>
<td>(0.23)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell</td>
<td>0.83 b</td>
<td>0.32 b c</td>
<td>4.02 b</td>
<td>1.41 b c</td>
<td>1.00 b</td>
<td>0.72 b</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>(0.88)</td>
<td>(0.26)</td>
<td>(1.85)</td>
<td>(0.28)</td>
<td>(0.05)</td>
<td></td>
</tr>
<tr>
<td>Large cell</td>
<td>0.94 b</td>
<td>0.46 b</td>
<td>3.48 b</td>
<td>2.01 b</td>
<td>0.81 b</td>
<td>0.57 b</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>(0.44)</td>
<td>(0.38)</td>
<td>(2.98)</td>
<td>(0.23)</td>
<td>(0.05)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.56 (0.15)</td>
<td>0.30 (0.17)</td>
<td>4.70 (2.17)</td>
<td>2.44 (1.48)</td>
<td>0.85 (0.23)</td>
<td>0.34 (0.23)</td>
</tr>
</tbody>
</table>

* Mean (SD); expressed as % of actin mRNA.

b Not different from other histological types; Wilcoxon rank sum test P > .05.

c Tumor significantly lower than normal; Wilcoxon rank sum test P < .05.

Table 3 Comparison of normal lung, lung tumors, and interindividual variation of levels of CYP2B7, CYP4B1, and OR mRNAs

<table>
<thead>
<tr>
<th></th>
<th>CYP2B7 Normal</th>
<th>CYP2B7 Tumor</th>
<th>CYP4B1 Normal</th>
<th>CYP4B1 Tumor</th>
<th>OR Normal</th>
<th>OR Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lung from</td>
<td>0.89 (0.74)*</td>
<td>0.39 (0.26)</td>
<td>4.03 (2.08)</td>
<td>1.69 (1.72)</td>
<td>0.88 (0.32)</td>
<td>0.65 (0.25)</td>
</tr>
<tr>
<td>lung cancer patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal lung from</td>
<td>1.05 (0.62)</td>
<td>1.51 (1.29)</td>
<td>0.98 (0.24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kidney donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung tumors</td>
<td>0.39 (0.26)</td>
<td>1.69 (1.72)</td>
<td>0.65 (0.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction of gene</td>
<td>2.3-fold b</td>
<td>2.4-fold</td>
<td>1.4-fold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expression in tumors</td>
<td>(14) c</td>
<td>33</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interindividual</td>
<td>30</td>
<td>64</td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>variation in normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lung</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Interindividual</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>variation in lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumors</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Mean (SD); expressed as % of actin mRNA.

b Mean of normal/mean of tumor.

c Highest/lowest detectable.

2B7 in human lung indicates that metabolism of xenobiotics in that organ is not merely a reflection of reactions occurring in the liver but may include unique catalytic pathways. The 2B7 joins other P450s already documented to be present in human lung including CYP1A1, CYP2F1, and CYP4B1. The mechanism for tissue-specific expression of genes encoding these P450s is under investigation.

The levels of both 2B7 and 4B1 mRNAs were higher than those of OR mRNA in normal lung. This result is consistent with the observation that one molecule of oxidoreductase can facilitate reduction of several molecules of P450. The ratio of total P450 proteins to OR is 15:1 in rat liver microsomal membranes (21). The levels of the three mRNAs tested exhibited significant interindividual variation in normal lung. Since the three genes are present as a single copy, different transcription and mRNA degradation rates influence observed variations. The 5.3-fold variation of OR mRNA level in normal human lung reported in this study is comparable with the variation of about 3-fold found in a group of normal adult livers and confirms that this gene is constitutively expressed in both organs (14). The high interindividual variability in levels of P450 mRNAs, as high as 33-fold (Table 3), may be due to environmental factors or genetic differences in regulatory mechanisms. The distribution of the levels of the three mRNAs in individual samples resembles the normal Gaussian distribution. The analysis of frequency distribution uncovered unimodal distribution of CYP2B7 and CYP4B1 mRNAs levels, suggestive of a lack of genetic polymorphism (Fig. 6). Further studies are necessary to elucidate if there is a genetic base for the variability observed in this survey.
S-transferase is considered one of the mechanisms of multidrug resistance exhibited by lung tumor cells (23).

Small cell lung cancer is an important type of lung neoplasm. It is characterized by a neuroendocrine differentiation phenotype and is associated with specific autocrine markers and chemosensitivity generally greater than that of non-small cell lung cancers (24). The levels of the CYP2B7, CYP4B1, and oxidoreductase mRNAs were the same among the major histological types of tumors represented in this study (Table 2). However, this observation cannot be extended to small cell lung cancer due to the limited number of RNA samples representing this histological type in our survey.

The biological importance of the statistically significant reduction of the three mRNAs in tumors as compared with normal lung remains unknown. We observed that the levels of the two P450 mRNAs are reduced in tumors to the same extent. Currently, we attribute this finding to chance rather than to a common, underlying, regulatory mechanism as there was no correlation between the levels of these two mRNAs in normal lung (data not shown). The reduced level of P450 mRNA in tumors is contrasted by a stable level of oxidoreductase mRNA, not only detectable in all neoplasms but not significantly lower in tumors than in normal lung. The steady level of expression of this gene agrees with the observation that the enzyme participates not only in reduction of P450s but in other reactions such as the NADPH-linked peroxidation of microsomal lipids (25) and the oxidative degradation of heme (26).

The extent of interindividual variations in the levels of the three mRNAs found in normal tissues were increased in the tumors. In addition to the genetic and environmental factors determining the mRNA levels in the normal lung, the tumors exhibit inherent genetic instability, consistent with multiple molecular alterations observed in all tumors and recently described in mouse lung neoplasms (27). The presence of CYP2B7 mRNA in most of the tumors tested is not reflected in the lung tumor cell lines, and it makes them rather poor models of neoplasms to be used in a drug discovery effort (20).

The full appreciation of the consequences of the reported levels of CYP2B7, CYP4B1, and CYPOR mRNAs in human lung awaits a detailed characterization of metabolic properties of these enzymes.

**Effects of Smoking.** The relationship between smoking and cancer is well documented. The susceptibility to form lung tumors may be associated with specific oxidation phenotypes including CYP1A1 present in human lung (28). Indeed, cigarette smoking induces this P450 in human lung (9). There are no studies reporting the influence of cigarette smoking on mRNA or protein levels of CYP2B7, CYP4B1, or OR. We examined our data for the evidence of induction of the levels of CYP2B7, CYP4B1, and CYPOR mRNAs by cigarette smoking but found no such association. This is in agreement with the known inducibility of rat CYP2B1 gene by phenobarbital but not by aromatic hydrocarbons. Phenobarbital can also increase the amount of OR mRNA and protein (21). Presently there is no information on inducibility of the CYP4B1 gene.

**REFERENCES**


Quantification of CYP2B7, CYP4B1, and CYPOR Messenger RNAs in Normal Human Lung and Lung Tumors

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