# Immunohistochemical Detection of Pulmonary Cytochrome P450IA and Metabolic Activities Associated with P450IA1 and P450IA2 Isozymes in Lung Cancer Patients

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The main polycyclic aromatic hydrocarbon-inducible cytochrome P450 was studied in lung tissue from 57 lung cancer patients by immunohistochemistry, using a monoclonal antibody (1-7-1) that recognizes P450IA1 and P450IA2 isozymes. The intensity of immunostaining was compared with the pulmonary activity of a P450IA1-dependent enzyme, aryl hydrocarbon hydroxylase (AHH), and with P450IA2-related metabolic activity estimated from the ratio of caffeine metabolites in urine. Immunostaining was not observed in peripheral lung tissue of nonsmokers or ex-smokers but was seen in the bronchiolar and alveolar epithelium of all patients who were smokers and had a peripheral carcinoma (16/16) and of 60% (10/17) of those who had a bronchial carcinoma. AHH activity was positively related to the intensity of immunostaining, and an almost 2-fold increase due to smoking was detected in the ratios of caffeine metabolites. These results demonstrate that tobacco smoke induces P450IA1 in the lung and probably P450IA2 in the liver, and suggest a role for certain metabolic phenotypes of P450IA1 in peripheral pulmonary carcinoma.

## Introduction

Tobacco smoke contains several classes of carcinogens; of these polycyclic hydrocarbons and aromatic amines may be preferentially metabolized by members of the P450 superfamily including P450IA1 and P450IA2 enzymes. P450IA1 is presumed to be important in pulmonary carcinogenesis, since it is involved in the biotransformation of polycyclic hydrocarbons to yield reactive intermediates that can bind to DNA (1-3). P450IA2 is mainly a hepatic enzyme that catalyzes the initial activation step of primary arylamines and is also involved in caffeine biotransformation (4). P450IA1 mRNA (CYP1A1 gene) is expressed in human lung (5), but the role of pulmonary P450IA2 in the metabolism of aromatic amines in tobacco smoke is regarded as negligible.

We have used immunohistochemistry previously to examine the presence and location of pulmonary cytochrome P450IA1 and P451A2 induced by tobacco smoking in lung cancer patients. As the monoclonal antibody used recognizes both P450IA1 and P450IA2, we use the term P450IA enzymes. The enzyme was found in the alveolar and bronchiolar epithelial cells of all patients who had a peripheral cancer, and in about half of those who had a bronchial cancer (6). The aim of this study was to confirm with a larger number of patients that the inducibility of P450IA differs between patients with different lung cancer types. The activity of a P450IA1-dependent enzyme, aryl hydrocarbon hydroxylase (AHH), determined in lung tissue homogenates, and P450IA2-related metabolic activity, which was estimated from the ratio of caffeine metabolites in urine from the same patients, were related to the immunohistochemical findings in the lung.

## Patients and Methods

Forty-nine male and eight female lung cancer patients were interviewed prior to lung surgery about their smok-

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ing habits, occupational history and use of alcohol and drugs. The age of the patients ranged from 35 to 78 years (mean, 60 years). Four of them were nonsmokers, 35 were current smokers, and 19 were ex-smokers among whom the time since stopping smoking ranged from 2 months to 48 years. Twenty-four-hour urine samples were collected from all patients at the hospital.

For immunohistochemistry and enzyme assays, fresh lung tissue was taken from surgical lobectomy or pulmectomy specimens and kept at −70°C until analyzed. The remaining surgical specimens were fixed with 10% formalin, and the histological samples were processed conventionally for light microscopy. The histological type and the point of origin of the tumors, whether in cartilaginous bronchi or in more peripheral airways, were examined. In three cases, the site of origin could not be determined because of the large size of the tumor. Thirty-one tumors were bronchial carcinomas: 25 squamous-cell carcinomas, 4 small-cell carcinomas, 1 large-cell carcinoma, and 1 adenocarcinoma; and 23 were peripheral: 18 adenocarcinomas, 4 squamous-cell carcinomas, and 1 large-cell carcinoma.

For immunohistochemistry, frozen sections were stained with a monoclonal antibody (Mab 1-7-1) raised against a methyl-cholanthrene-inducible rat cytochrome P450 isozyme (provided by H. Gelboin and S. S. Park, National Cancer Institute, Bethesda, MD) using an avidin-biotin-peroxidase method (6,7). Immunostaining of peripheral lung tissue was classified into three grades: no staining, positive, and strongly positive (if a large number of both bronchiolar and alveolar epithelial cells were stained).

To measure AHH activity, the lung samples were homogenized and the microsomal fraction was separated. AHH activity was determined by the fluorimetric method of Nebert and Gelboin (8), which has a detection limit of 0.05 pmole 3-hydroxybenzo[a]pyrene $[min]^{-1}[mg]^{-1}$ protein.

A high-performance liquid chromatographic method was used to quantify the urinary levels of caffeine (137X) and its metabolites, i.e., 1,7-dimethylxanthine (17X), 1-methylxanthine (1X), 1-methyluric acid (1U), 1,7-dimethyluric acid (17U) and 5-acetylamino-6-amino-3-methyluracil (AAMU), as described previously (9,10). The ratio 17X/137X has been measured in 30 patients and that of AAMU + 1U + 1X/17U in 37 patients to date. These ratios reflect P450IA2-dependent metabolic activities, mostly in the liver.



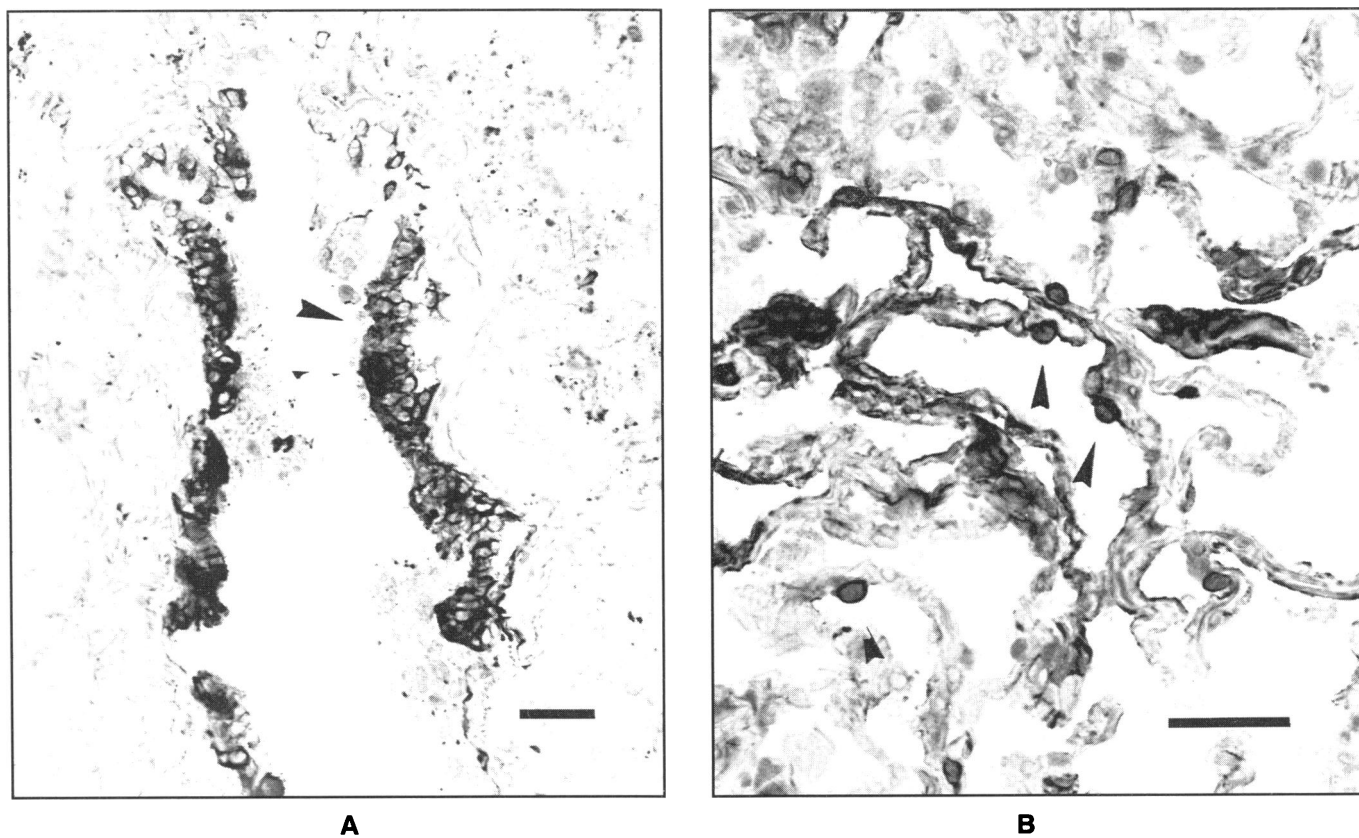**A**                                                                  **B**

FIGURE 1. Immunostaining of frozen sections from peripheral lung tissue shows cytochrome P450IA in bronchiolar (A) and alveolar epithelium (B). In a longitudinal section of a bronchiolus, strongly immunopositive columnar ciliated epithelial cells can be seen (arrowheads, A). At the center of B, immunopositive alveolar type I epithelial cells line alveolar spaces; some positive type II cells are also visible (arrowheads, B). Immunoperoxidase, hematoxylin counterstain. Bar = 40 μm.

# Results and Discussion

Detection of pulmonary cytochrome P450IA by immunohistochemical staining requires induction by smoke constituents, as this cytochrome cannot be detected in lung tissue from nonsmokers and ex-smokers (6) and the activity of AHH in lung tissue vanishes within 2 months after stopping smoking (11). Peripheral lung tissue from smokers showed patchy immunopositive areas localized in bronchiolar and in type I and type II alveolar epithelial cells; in strongly positive cases, they were also found in vascular endothelium (Fig. 1). AHH activity in lung tissue homogenate was positively related to the intensity of immunohistochemical staining in lung tissue from the same patients (Fig. 2A). The ratios of caffeine metabolites were less clearly associated with P450IA immunostaining and smoking: on a plot of the 17X/137X caffeine ratios, no correlation was apparent (not shown), but a 1.8-fold effect on the AAMU + 1X + 1U/17U ratio due to smoking ($p <$ 0.01) was seen with some positive trend with the intensity of immunostaining (Fig. 2B). These results demonstrate the inducing effect of tobacco smoke on P450IA1 in the lung and on P450IA2 probably in the liver of smokers (4,12). Further molecular analyses are required to determine whether CYP1A2 and other CYPs are expressed in the lungs of smokers.

Peripheral lung tissue was P450IA-immunopositive in all patients who smoked and had a peripheral lung cancer (16/16) but in only about 60% (10/17) of those who had a bronchial cancer. This result is consistent with the findings in the first 25 patients published earlier. We also observed much weaker P450IA immunostaining in bronchial than in peripheral airway epithelium in both cancer types (6). Metabolic transformation of polycyclic hydrocarbons by P450IA thus seems to take place in the same part of the airways and in the same cell types as those in which peripheral carcinomas arise. Our finding of inducible cytochrome P450IA in all patients with peripheral pulmonary carcinoma and the location of this cytochrome suggest that these patients may be especially prone to cancer induced by tobacco smoking and the risk is increased in certain metabolic phenotypes.
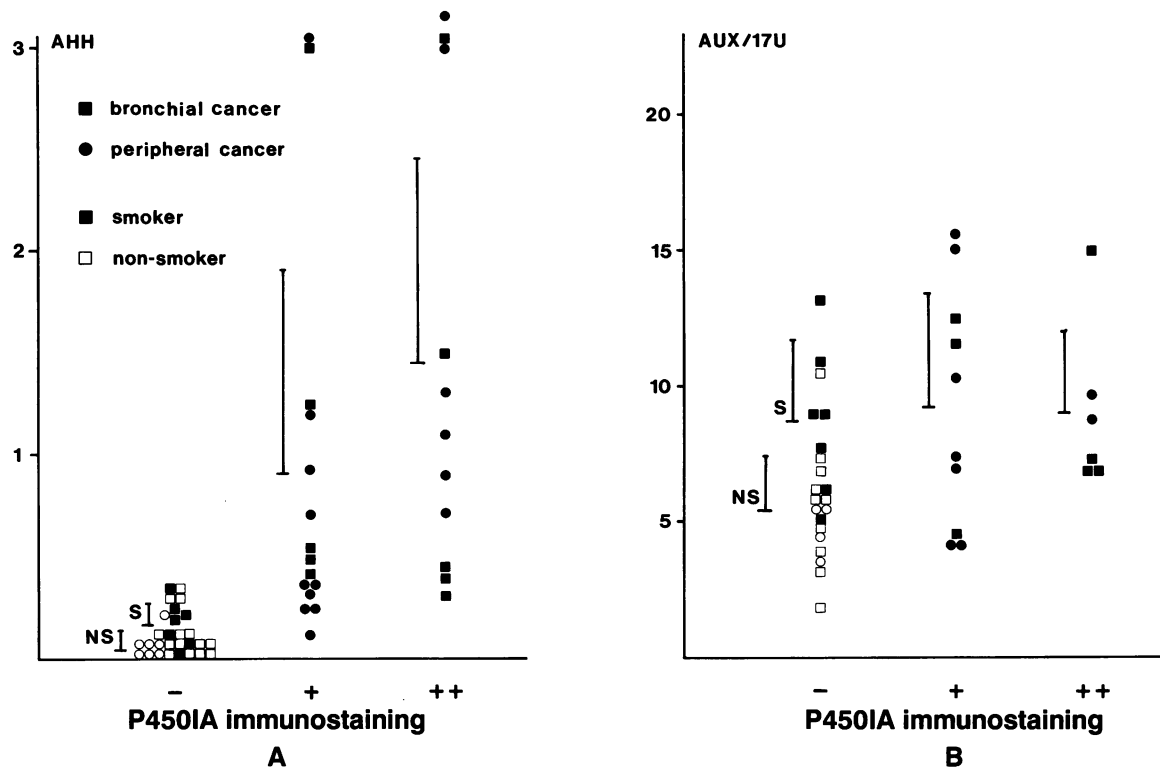
FIGURE 2. Immunostaining of peripheral lung tissue compared with aryl hydrocarbon hydroxylase (AHH) activity (pmole [mg]$^{-1}$[min]$^{-1}$) in lung tissue homogenate (A) and with the urinary caffeine metabolite ratio AAMU + 1U + 1X (AUX)/17U (B). In the first column of both parts of the figure, the mean values with standard deviations are indicated separately for current smokers (S) and nonsmokers including ex-smokers (NS). ( − ), no staining; ( + ), positive; ( + + ), strongly positive.

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