Evidence-Based Prevention (EBP): A Review of Cytochrome P450 Expression in the Bronchial Epithelium and New Approach to Lung Cancer Prevention*

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Abstract

The number of fatalities in Japan attributable to lung cancer exceeded 50000 in 2001. It is socially desirable that various markers, which can be utilized for the prevention of lung cancer, be established. We believe that smoking or exposure to carcinogens in air induces mutations in bronchial and alveolar epithelia, leading to the development of lung cancer. It would be useful to have markers of individual differences in susceptibility to chemical carcinogen-induced lung cancer 1) to identify genetic polymorphisms of enzymes metabolizing chemical carcinogens and 2) to investigate the expression of enzymes metabolizing chemical carcinogens. In this paper, we review CYP expression in the bronchial epithelium. CYP1, CYP2 and CYP3 are expressed in the bronchial epithelium. We also show the relationship between the genetic polymorphisms of cytochrome P450 (CYP) and a person's susceptibility to chemical carcinogen-induced lung cancer. We demonstrate the relationship between cigarette consumption and the CYP expression profile in the bronchial epithelium. To maintain and promote public health, we must apply evidence, such as CYP polymorphisms and CYP profiles to disease prevention and also to aggressively advance evidence-based prevention (EBP) of lung cancer.

Key words: cytochrome P450 (CYP), evidence-based prevention (EBP), lung cancer, polymorphism, bronchial epithelium

Introduction

The number of fatalities in Japan attributable to lung cancer exceeded 50000 in 2001. Fatalities caused by lung cancer account for the largest number of cancer deaths. Lung cancer is an intractable disease and over one-half of those with lung cancer die (1). It is socially desirable that markers that can be utilized for the prevention of lung cancer be established (2–13).

It seems that smoking or exposure to carcinogens in air induces mutations in the bronchial and alveolar epithelia, leading to the development of lung cancer. Genes such as *p53* are targeted by genetic alterations, genetic surveillance and DNA repair are disrupted, and genetic alterations continue to accumulate at an accelerated pace, leading to a sequential multistep carcinogenesis (14, 15). Smoking is a major cause of lung cancer, but not all smokers develop lung cancer. This observation has led to the belief that individual differences exist in susceptibility to chemical carcinogen-induced lung cancer (16). It would be useful to have markers of individual differences in susceptibility to chemical carcinogen-induced lung cancer 1) to identify genetic polymorphisms of enzymes metabolizing chemical carcinogens and 2) to investigate the expression of enzymes metabolizing chemical carcinogens in the bronchial epithelium.

In 1991, the concept of evidence-based medicine (EBM) was introduced by Guyatt and published in the JAMA (17), and this has greatly influenced current medical care and education (18). We believe that we can grasp individually tailored

Abbreviation: cytochrome P450, CYP; single-nucleotide polymorphism, SNP; evidence-based prevention, EBP.

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disease prevention based on markers of individual differences in susceptibility to chemical carcinogen-induced lung cancer using evidence-based prevention (EBP) similarly to EBM (19). In this paper, we present our studies, in which we investigated the relationship between genetic polymorphisms of cytochrome P450 (CYP) and susceptibility to chemical carcinogen-induced lung cancer. We review CYP expression in the bronchial epithelium. We also suggest the relationship between cigarette consumption and CYP expression in the bronchial epithelium.

Metabolic activation of carcinogens by cytochrome P450

The relative risk of lung cancer in Japanese smokers compared with nonsmokers is approximately five times greater for men and three times greater for women, indicating that compared with other risk factors, smoking is overwhelmingly involved in lung cancer development (18, 20). Taking benzo[a]pyrene and nitrosamine as examples, which are carcinogens that are present in cigarette smoke, Figure 1 shows the pathways from their metabolic activation by CYP to carcinogenesis. Benzo[a]pyrene and nitrosamine undergo metabolic activation by the phase I enzymes CYP1A1, CYP2A6 and CYP2E1 (21–23). Carcinogens that are activated by CYP move into the nucleus and bind to DNA to form DNA adducts that exhibit strong carcinogenicity. It is thought that if the adducts target the p53 gene in particular, which is a tumor suppressor gene related to cell repair and apoptosis, genetic alterations including mutations cause errors in DNA repair at an accelerated pace, leading to carcinogenesis (4, 14, 24, 25). Carcinogens that are activated by phase I enzymes are mostly converted to inactive metabolites by the phase II enzyme glutathione S-transferase

(GST), particularly the Mu class (GSTM1) (26).

Cytochrome P450 polymorphism and susceptibility to chemical carcinogen-induced lung cancer

A genetic polymorphism is defined as the occurrence of variants at a gene locus at a frequency of greater than 1% in the same population in a base sequence of about 3 billion, and is called a single-nucleotide polymorphism (SNP) when one base is replaced by an other base (27). Currently, one SNP locus occurs per approximately 1,000 nucleotides in an individual and it is thought that combinations of 3 to 10 million SNP loci that are scattered throughout the genome contribute to individual differences. Researchers are considering apply evidence-based genetic polymorphism analysis to 1) predicting disease development, 2) identifying disease-related genes and developing drugs that target them, and 3) predicting and reducing adverse drug effects (27).

SNP loci also exist within the CYP gene, resulting in individual differences in enzyme properties (structure), the level of transcription and ultimately enzyme activity (28). Smokers with a high CYP1A1 activity and low GST activity are expected to have a high risk of lung cancer, and a previous study showed that polymorphisms of CYP1A1 and GST are associated with this risk (21, 22, 29). On the other hand, the enzymes CYP2E1 and CYP2A6 are highly expressed in the bronchial epithelium (30). We reported that a high CYP2E1 activity in smokers might be associated with the development of lung cancer arising from the bronchial epithelium (23). In any event, since the induction of enzyme expression attributable to an increased exposure to carcinogens and CYP differs among racial groups (21, 22), the susceptibility of individuals to lung cancer is thought to be difficult to diagnose when only using a single-enzyme SNP (19).

Fig. 1 Metabolic pathways that utilize carcinogens. Carcinogens introduced into cells are metabolized by phase I enzymes and detoxified by phase II enzymes. The carcinogens are activated by phase I enzymes to DNA-binding metabolites. The metabolites then interact with chromosomal DNA and form DNA adducts, which may produce mutations and trigger cell death or the development of cancer.

Cytochrome P450 expression in bronchial epithelium

Genetic studies can be used to reveal not only private information, but also the genetic disposition of blood relatives. Therefore, the results of genetic studies may cause ethical, legal and social problems. Enzymes metabolizing chemical carcinogens are expressed because of not only their genotype, but also their induction by the intake of carcinogenic food and smoking (18). Several CYPs are expressed in extrahepatic tissue although CYPs are mainly expressed in liver (18). We summarized CYP expression in the bronchial epithelium in Table 1. CYP1, CYP2 and CYP3 are expressed in the bronchial epithelium (Table 1), but the relationship between lung cancer risk factors such as smoking and CYP expression in the bronchial epithelium has not been clarified.

Thus, we investigated the relationship between cigarette consumption and the CYP expression profile in the bronchial epithelium; we have applied for a patent for this profile (2004 publication of patent application No. 279385). CYP1A1, CYP2A6 and CYP2E1 may be induced in the bronchial epithelium

when the bronchial epithelium is exposed to carcinogens via smoking. CYP1A1, CYP2A6 and CYP2E1 in turn activate carcinogens, such as benz[a]pyrene. The carcinogens activated by CYP1A1, CYP2A6 and CYP2E1 work as DNA adducts and the DNA adducts cause various DNA mutations. The accumulations of DNA mutations in the bronchial epithelium may result in epithelial neoplasm (Fig. 2).

The immunohistochemical staining for CYP1A1, CYP2A6 and CYP2E1 was performed on 3 µm sections of resected human lung tissue embedded in paraffin. We defined positivity for the enzymes in the case when more than 10% of the bronchial epithelium was stained (CYP-negative case tissue sections, 10% > positivity; CYP-positive case tissue sections, 10% ≤ positivity). The positivity rate for CYP1A1, CYP2A6 and CYP2E1 in the bronchial epithelium increased in relation to the increase with lifetime cigarette consumption (manuscript submitted). Figure 3 shows the results of an examination by sputum cytodiagnosis of the bronchial epithelium of a healthy subject. The column epithelium with cilia (bronchial epithelium) can be recognized by Papanicolaou staining. For CYP2A6

Table 1 Expression of cytochrome P450 (CYP) in bronchial epithelium

Cytochrome P450	Expression cell	Induction factor	Reference
CYP1A1 CYP1B1	Bronchial epithelium	Smoking Female Benzo[a]pyrene	Hukkanen, et al., Berge G, et al., and Port JL, et al. ^(32–34)
CYP2A6 CYP2B6 CYP2B7 CYP2E1 CYP2F1 CYP2J2	Bronchial epithelium Bronchial alveolar Alveolar macrophage Clara cell Type II pneumocyte	Ethanol	Crawford, et al. Hukkanen, et al., and Runge, et al. ^(30, 32, 35)
CYP3A4 CYP3A5	Bronchial epithelium Bronchial alveolar Bronchial glands Alveolar macrophage	Smoking	Raunio H, et al. ^(36, 37)

Superscripts indicate the reference numbers.

Fig. 2 CYP metabolic activation and induction is related to carcinogenesis. CYP1A1, CYP2A6 and CYP2E1 are induced in the bronchial epithelium when the bronchial epithelium is exposed to carcinogen via smoking. They activate the carcinogens. The activation causes various DNA mutations. The accumulation of DNA mutations in the bronchial epithelium results in epithelial neoplasm.

Fig. 3 Examination of bronchial epithelium by sputum cytodiagnosis. Column epithelium with cilia (bronchial epithelium) can be identified by Papanicolaou staining. For CYP2A6 staining a mouse monoclonal antibody (Daiichi Pure Chemicals Co., Ltd., Tokyo) was used. The risk of lung cancer may be low when the bronchial epithelium is negatively stained for several CYPs and may be high when positively stained.

staining, a mouse monoclonal antibody (Daiichi Pure Chemicals Co., Ltd., Tokyo) was used as described previously (31). The risk of lung cancer may be low when the bronchial epithelium is negatively stained for several CYPs, but may be high when it is positively stained. Further studies will be needed to confirm the relationship between the CYP expression profile and the risk of lung cancer. Based on enzyme expression profiling, it should be possible to accurately predict the risk of cancer development and formulate individually tailored plans for cancer prevention.

Conclusions

The results of genetic and epigenetic studies may cause ethical, legal and social problems. It is indispensable that individual dignity be respected and that appropriate research be carried out on the bases of appropriate guidelines. The Human Genome Project has estimated that the number of human genes is about 30,000. The project has led to a rapid increase in the amount of available information about genes and the develop-

ment of innovative techniques for simultaneously analyzing thousands to tens of thousands of genes. Thus, a molecular-epidemiological approach to the analysis of the risk of lung cancer and other diseases is entering a dramatically dynamic phase. At present, in a conscious effort to maintain and promote public health, we must apply evidence, such as that on CYP polymorphisms and CYP profile, to disease prevention and also to aggressively advance EBP of lung cancer.

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References

- (1) Uramoto H, Nakanishi R, Fujino Y, Imoto H, Takenoyama M, Yoshimatsu T, et al. Prediction of pulmonary complications after a lobectomy in patients with non-small cell lung cancer. *Thorax*. 2001;56:59–61.
- (2) Osaki T, Mitsudomi T, Oyama T, Nakanishi R, Yasumoto K. Serum level and tissue expression of c-erbB-2 protein in lung adenocarcinoma. *Chest*. 1995;108:157–162.
- (3) Gu CD, Osaki T, Oyama T, Inoue M, Kodate M, Dobashi K, et al. Detection of micrometastatic tumor cells in pN0 lymph nodes of patients with completely resected nonsmall cell lung cancer; Impact on recurrence and survival. *Ann Surg*. 2001;233:133–139.
- (4) Osaki T, Oyama T, Inoue M, Gu CD, Kodate M, Aikawa M, et al. Molecular biological markers and micrometastasis in resected non-small-cell lung cancer. Prognostic implications. *Jpn J Thorac Cardiovasc Surg*. 2001;49:545–551.
- (5) Osaki T, Oyama T, Gu CD, Yamashita T, So T, Takenoyama M, et al. Prognostic impact of micrometastatic tumor cells in the lymph nodes and bone marrow of patients with completely resected stage I non-small-cell lung cancer. *J Clin Oncol*. 2002;20:2930–2936.
- (6) Oyama T, Kawamoto T, Matsuno M, Osaki T, Matsumoto A, Isse T, et al. A case-case study comparing the usefulness of serum trace elements (Cu, Zn and Se) and tumor markers (CEA, SCC and SLX) in non-small cell lung cancer patients. *Anticancer Res*. 2003;23:605–612.
- (7) Gu CD, Oyama T, Osaki T, Li J, Takenoyama M, Izumi H, et al. Low expression of polypeptide GalNAc N-acetylglucosaminyl transferase-3 in lung adenocarcinoma: Impact on poor prognosis and early recurrence. *Br J Cancer*. 2004;

- 90:436–442.
- (8) Uramoto H, Sugio K, Oyama T, Nakata S, Ono K, Morita M, et al. Expression of deltaNp73 predicts poor prognosis in lung cancer. *Clin Cancer Res.* 2004;10:6905–6911.
 - (9) Iwata T, Uramoto H, Sugio K, Fujino Y, Oyama T, Nakata S, et al. A lack of prognostic significance regarding DeltaNp63 immunoreactivity in lung cancer. *Lung Cancer.* 2005;50:67–73.
 - (10) Oyama T, Osaki T, Baba T, Nagata Y, Mizukami M, So T, et al. Molecular genetic tumor markers in non-small cell lung cancer. *Anticancer Res.* 2005;25:1193–1196.
 - (11) Uramoto H, Sugio K, Oyama T, Nakata S, Ono K, Yoshimatsu T, et al. Expression of endoplasmic reticulum molecular chaperone Grp78 in human lung cancer and its clinical significance. *Lung Cancer.* 2005;49:55–62.
 - (12) Uramoto H, Sugio K, Oyama T, Ono K, Sugaya M, Yoshimatsu T, et al. Epidermal growth factor receptor mutations are associated with gefitinib sensitivity in non-small cell lung cancer in Japanese. *Lung Cancer.* 2005 (in press).
 - (13) Yoshimatsu T, Uramoto H, Oyama T, Yashima Y, Gu C, Morita M, et al. Y-box-binding protein-1 expression is not correlated with p53 expression but with proliferating cell nuclear antigen expression in non-small cell lung cancer. *Anticancer Res.* 2005;25:3437–3443.
 - (14) Mitsudomi T, Oyama T, Kusano T, Osaki T, Nakanishi R, Shirakusa T. Mutations of the p53 gene as a predictor of poor prognosis in patients with non-small cell lung cancer. *J Natl Cancer Inst.* 1993;85:2018–2023.
 - (15) Mitsudomi T, Oyama T, Nishida K, Ogami A, Osaki T, Sugio K, et al. Loss of heterozygosity at 3p in non-small cell lung cancer and its prognostic implication. *Clin Cancer Res.* 1996;2:1185–1189.
 - (16) Kawamoto T, Isse T, Kumugita N, Yang M, Kitagawa K, Suenaga R, et al. Effects of genetic polymorphism of drug metabolizing enzymes on smoking and drinking. *J UOEH.* 2003;25:97–106.
 - (17) Evidence-Based Medicine Working Group. Evidence-based medicine. A new approach to teaching the practice of medicine. *JAMA.* 1992;268:2420–2425.
 - (18) Oyama T, Kawamoto T, Matsumoto A, Isse T, Ozaki S, Yasumoto K. Evidence based prevention (EBP): Evidence-based approach to prevention of lung cancer by application of cytochrome 2E1 polymorphism. *J UOEH.* 2002;24:413–421.
 - (19) Oyama T, Matsumoto A, Isse T, Kim Y-D, Ozaki S, Osaki T, et al. Evidence-based prevention (EBP): Approach to lung cancer prevention based on cytochrome 1A1 and cytochrome 2E1 polymorphism. *Anticancer Res.* 2003;23:1731–1738.
 - (20) Sobue T, Yamamoto S, Hara M, Sasazuki S, Sasaki S, Tsugane S, et al. Cigarette smoking and subsequent risk of lung cancer by histologic type in middle-aged Japanese men and women: the JPHC study. *Int J Cancer.* 2002;99:245–251.
 - (21) Oyama T, Mitsudomi T, Kawamoto T, Ogami A, Osaki T, Kodama Y, et al. Detection of CYP1A1 gene polymorphism using designed RFLP and distributions of CYP1A1 genotypes in Japanese. *Int Arch Occup Environ Health.* 1995;67:253–256.
 - (22) Oyama T, Kawamoto T, Mizoue T, Nishida K, Osaki T, Sugio K, et al. p53 mutations of lung cancer are not significantly affected by CYP1A1 or GSTM1 polymorphisms. *Int J Oncol.* 1997;11:305–309.
 - (23) Oyama T, Kawamoto T, Mizoue T, Sugio K, Kodama Y, Mitsudomi T, et al. Cytochrome P450 2E1 polymorphism as a risk factor for lung cancer: in relation to p53 gene mutation. *Anticancer Res.* 1997;17:583–587.
 - (24) Oyama T, Osaki T, Mitsudomi T, Ogawa R, Nakanishi R, Sugio K, et al. p53 alteration, proliferating cell nuclear antigen, and nucleolar organizer regions in thymic epithelial tumors. *Int J Mol Med.* 1998;1:823–826.
 - (25) Oyama T, Osaki T, Nose N, Ichiki Y, Inoue M, Imoto H, et al. Evaluations of p53 immunoreactivity, nucleolar organizer regions, and proliferating cell nuclear antigen in non-small cell lung carcinoma. *Anticancer Res.* 2000;20:505–510.
 - (26) Kawajiri K, Eguchi H, Nakachi K, Sekiya T, Yamamoto M. Association of CYP1A1 germ line polymorphisms with mutations of the p53 gene in lung cancer. *Cancer Res.* 1996;56:72–76.
 - (27) Oyama T, Kagawa N, Kim Y-D, Matsumoto A, Isse T, Kawamoto T. Lung cancer and CYP1A1 or GSTM1 polymorphism. *Environ Health Prev Med.* 2003;7:230–234.
 - (28) Ingelman-Sundberg M. Genetic susceptibility to adverse effects of drugs and environmental toxicants. The role of the CYP family of enzymes. *Mutat Res.* 2001;482:11–19.
 - (29) Goto I, Yoneda S, Yamamoto M, Kawajiri K. Prognostic significance of germ line polymorphisms of the CYP1A1 and glutathione S-transferase genes in patients with non-small cell lung cancer. *Cancer Res.* 1996;56:3725–3730.
 - (30) Crawford EL, Weaver DA, DeMuth JP, Jackson CM, Khuder SA, Frampton MW, et al. Measurement of cytochrome P450 2A6 and 2E1 gene expression in primary human bronchial epithelial cells. *Carcinogenesis.* 1998;19:1867–1871.
 - (31) Oyama T, Isse T, Kagawa N, Kinaga T, Kim YD, Morita M, et al. Tissue-distribution of aldehyde dehydrogenase 2 and effects of the ALDH2 gene-disruption on the expression of enzymes involved in alcohol metabolism. *Front Biosci.* 2005;10:951–960.
 - (32) Hukkanen J, Pelkonen O, Hakkola J, Raunio H. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit Rev Toxicol.* 2002;32:391–411.
 - (33) Berge G, Mollerup S, OVrebo S, Hewer A, Phillips DH, Eilertsen E, et al. Role of estrogen receptor in regulation of polycyclic aromatic hydrocarbon metabolic activation in lung. *Lung Cancer.* 2004;45:289–297.
 - (34) Port JL, Yamaguchi K, Du B, De Lorenzo M, Chang M, Heerdt PM, et al. Tobacco smoke induces CYP1B1 in the aerodigestive tract. *Carcinogenesis.* 2004;25:2275–2281.
 - (35) Runge DM, Stock TW, Lehmann T, Taege C, Bernauer U, Stolz DB, et al. Expression of cytochrome P450 2E1 in normal human bronchial epithelial cells and activation by ethanol in culture. *Arch Toxicol.* 2001;75:335–337.
 - (36) Raunio H, Hakkola J, Hukkanen J, Lassila A, Paivarinta K, Pelkonen O, et al. Expression of xenobiotic-metabolizing CYPs in human pulmonary tissue. *Exp Toxicol Pathol.* 1999;51:412–417.
 - (37) Raunio H, Hakkola J, Pelkonen O. Regulation of CYP3A genes in the human respiratory tract. *Chem Biol Interact.* 2005;151:53–62.