Lung Cancer and *CYP1A1* or *GSTM1* Polymorphisms

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Abstract

Most chemical carcinogens are metabolized and activated *in vivo* by phase I enzymes including the microsomal cytochromes P450 and epoxide hydroxylases. The carcinogens and their metabolites are detoxified by phase II enzymes that include various transferases such as glutathione-S-transferases (GST). Increasing numbers of studies have demonstrated the association of the polymorphisms in *GSTM1* (a member of GST) and *CYP1A1* genes with the susceptibility to lung cancer. Subsequently, the polymorphisms appear to be important biomarkers that provide information for assessment of exposure and total burden of environmental carcinogens. Therefore, the investigation of the polymorphisms in these genes will provide information not only for the prediction of individual cancer risk but also for the prevention of cancer. In this review, we will summarize the polymorphisms in the *GSTM1* and *CYP1A1* genes and their relation to lung cancer susceptibility.

Key words: cytochrome p4501A1 gene (*CYP1A1*) polymorphism, glutathione S-transferase μ gene (*GSTM1*) polymorphism, *p53* gene mutation, non-small-cell lung cancer (NSCLC), survival curve

Introduction

Cancer may primarily be a genetic disease. Cancerization contains multistage processes involving the accumulation of genetic changes. An accumulation of DNA damage enables clonal expansion of abnormal cells from a tumor cell. Cancer genes can be classified as caretaker genes and gatekeeper genes (1). Caretaker genes affect DNA repair, activation and detoxification of carcinogens. Gatekeeper genes affect cell cycle control and DNA replication. Dysfunction of caretaker genes may result in the increased probability of mutations. When mutations occur in gatekeeper genes, such as *p53*, the normal cell cycle could be interrupted. Such mutations, therefore, could cause the formation of cancer cells.

Procarcinogens enter the human body from a variety of sources, including diet, tobacco smoking, and industrial dust. Most chemical carcinogens require metabolic activation *in vivo* to trigger the development of cancer. Multiple steps of the metabolisms are generally classified into two phases, phase I and phase II steps. Cytochrome P450 families and epoxide hydroxylases are

the major members of phase I enzymes that introduce reactive groups into carcinogens to increase hydrophilicity and to produce reactive intermediates for the subsequent conjugation reaction. Glutathione-S-transferases, N-acetyltransferases, and sulfotransferases are the major enzymes involved in the phase II reactions that generally conjugate water-soluble moieties and render the reactive intermediates harmless. The reactive intermediates may interact with DNA to form DNA adducts that may eventually produce somatic mutations and trigger carcinogenesis. Therefore, individuals with high phase I activity and low phase II activity may have a higher risk of DNA damage.

The role of genetic factors in determining human susceptibility to the carcinogenic effects of environmental agents has been intensively investigated in molecular epidemiology. These genetic studies developed several types of biomarkers that are useful in understanding casual mechanisms of carcinogenesis (2). These biomarkers include markers of exposure (e.g., serum levels of nutrients), DNA damage (e.g., carcinogen-DNA adducts), early pathobiological effects (e.g., mutations in tumor suppressor genes, structural changes in chromosomes or changes in morphology) and inherited susceptibilities (inheritance of germ line mutations and genetic polymorphisms). These biomarkers can be modified by the individual variation of phase I and phase II activities, which may be caused by the highly frequent (>1%) genetic polymorphisms associated with phase I and phase II enzymes. Thus, the investigations of genetic polymorphisms in genes encoding phase I and phase II enzymes are important to understand the high degree of individual variability in cancer susceptibility. In this review, we discuss the

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Abbreviations: CYP1A1, Cytochrome p4501A1 gene; GSTM1, glutathione S-transferase μ gene; NSCLC, non-small-cell lung cancer; SNP, single nucleotide polymorphism.

polymorphisms in *CYP1A1* and *GSTM1* genes and their association with susceptibility to lung cancer.

Carcinogen metabolism and interindividual variation

Epidemiologic studies reveal that cancer risks differ among human populations. The differences among individuals are associated with life style, diet, environment, gender, race, and ethnicity. It is also true that only some people develop cancer in a population with similar exposure to carcinogens. People appear to have inherited traits that affect cancer risk (3). Variations in chromosomal DNA sequences are implicated in some differences in carcinogen

metabolism. A single base change, “single nucleotide polymorphism (SNP)”, may result in the production of a mutant enzyme with an amino acid substitution, that might substantially affect the metabolic activity of the enzyme (4).

As shown in Fig. 1, carcinogens exposed to cells are metabolized, and subsequently activated or discharged. Cytochromes P450, families of monooxygenases, are typical members of phase I enzymes that convert chemical carcinogens into DNA-binding intermediates (Table 1) (5). There are also several families in phase II enzymes that detoxify and discharge carcinogens (Table 1). Carcinogen-DNA adducts produced in oncogenes or tumor suppressor genes often yield mutations in these genes, which may lead to cell death or cancerization when DNA repair function can not rescue the DNA damage (Fig. 1).

Polymorphisms in the *CYP1A1* gene associated with carcinogen metabolism and cancer risk

Several different genetic polymorphisms in cytochrome P4501A1 gene (*CYP1A1*) have been identified in the human population (see <http://www.imm.ki.se/CYPalleles/cyp1a1.htm> for *CYP1A1* allele nomenclature). The *CYP1A1* *MspI* mutation (*CYP1A1**2A) is a point mutation in the 3'-noncoding region. The *I462V* mutation (*CYP1A1**2C) has an amino acid substitution of Ile at position 462 to Val. The *CYP1A1**2B variant contains both *I462V* and *MspI* mutations. The Thr461Asn mutation (*CYP1A1**4) has an amino acid substitution of Thr at position 461 to Asn. Individuals with homozygous mutant types of *MspI* and *I462V* have been shown to have increased susceptibility to lung cancer (6).

Although both wild-type and the mutant *I462V* were expressed in yeast in our laboratory, no differences between the two enzymes were found in *K_m* or *V_{max}* for O-dealkylation of ethoxyresorufin and 3-hydroxylation of benzo(a)pyrene (BaP). The data suggested that the *Ile462Val* mutation in human *CYP1A1* might not be functionally important. Utilizing a baculovirus system, we also expressed wild-type (*CYP1A1**1A), *I462V* (*CYP1A1**2C), and T461N (*CYP1A1**4) with the human NADPH-P450 reductase. The complete phase I metabolites from BaP and BaP-7,8-dihydrodiol were analyzed by HPLC. The relative total conversions of BaP compared with wild-type enzyme (100%) were

Fig. 1 The metabolic pathways of carcinogen.

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 DNA-binding metabolites interact with chromosomal DNA and form DNA adducts, which may produce mutations and trigger cell death or the development of cancer.

Table 1 Cytochrome P450, detoxification enzymes, and carcinogen substrates

Cytochrome P450	Chemical carcinogen substrates
P450 1A1	Polycyclic hydrocarbons
P450 1A2	Arylamines & heterocyclic amines
P450 1B1	Polycyclic hydrocarbons and arylamines
P450 2A6	N-Nitrosamines and aflatoxins
P450 2B6	Nitrosamines and aflatoxins
P450 2C9	Polycyclic hydrocarbons
P450 2E1	N-Nitrosamines, benzene, styrene, urethane, ethanol and Butadiene
P450 3A3	Aflatoxins, polycyclic hydrocarbons, aryl aromatic amines, N-nitrosamines
P450 3A4	Aflatoxins, polycyclic hydrocarbons, aryl aromatic amines
P450 17	Estrogen
Detoxification enzymes	Chemical carcinogen substrates
Glutathion S-transferases	polycyclic aromatic hydrocarbons, aflatoxins, etc.
N-acetyl-transferases	aryl aromatic amines, heterocyclic amines, arylamines, etc
Epoxide hydrolases	polycyclic aromatic hydrocarbons, styrene, etc
Sulfotransferases	aryl aromatic amines, polycyclic aromatic hydrocarbons, etc

approximately 50% for *CYP1A1**2C and approximately 70% for *CYP1A1**4. Km values for all metabolites with *CYP1A1**2C were significantly lower than with wild type enzyme (e.g. BaP-7,8-diol formation: 13.8 μ M for wild-type, 3.5 μ M for *CYP1A1**2 and 7.7 μ M for *CYP1A1**4). Addition of epoxide hydrolase markedly increases the relative diol-to-phenol activities by all three variants. However, *CYP1A1**4 exhibits the greatest efficiency to produce diol species. Each variant produced the diol epoxides from BaP-7,8-dihydrodiol. *CYP1A1**1A catalyzed the epoxidation reaction at the rate of 10.4 pmol/min/pmol P450, the greatest total rate for 7,8-diol metabolites followed by *CYP1A1**2C (7.2 pmol/min/pmol P450) and *CYP1A1**4 (5.5 pmol/min/pmol P450). All enzyme variants produced about three fold more diol epoxide 2-derived metabolites than diol epoxide 1-derivatives, whereby both rare allelic variants exhibited significantly increased formation of diol epoxide 2. This study showed that the three *CYP1A1* variants had different enzyme kinetic properties to produce both the diol metabolites from BaP and the ultimate mutagenic species diol epoxide 2 from BaP-7,8-dihydrodiol. Although the decreased catalytic activities of the mutants are not directly correlated with the increased production of active carcinogens, the delayed metabolisms may result in an accumulation of the carcinogens that induce phase I enzymes via arylhydrocarbon receptor, which may be associated with a higher production of active carcinogens.

Polymorphisms in glutathion-S-transferases

Five classes of soluble glutathion-S-transferases (GSTs) are known in humans, including α (A), μ (M), π (P), τ (T), and ξ (Z) with various subfamilies for each class (i.e. A1–A4, M1–M5, P1, T1–T2, and Z1) (7). Numerous polymorphisms occur in the genes encoding GSTs (8, 9). Of those, one polymorphism in *GSTM1* gene results in nonfunctional, deleted alleles. The homozygous null genotype of *GSTM1* (*GSTM1*; –/–) has been implicated in an increased risk for lung cancer (10).

In IARC Scientific Publications, the odds ratio (OR) above 1.0 carried by the *GSTM1*-null polymorphism was obtained in 17 studies among 21 studies on *GSTM1* and lung cancer (10). A meta-OR=1.34 (1.21–1.48) in *GSTM1*-null polymorphism was obtained in the results of meta-analysis on the 21 studies. Although four Japanese case-control studies also reported a meta-OR=1.73 (1.30–2.31) for homozygous mutant types of *MspI* in the *CYP1A1* gene, there were few case-case studies investigating the prevalence or the mortality of *GSTM1* and *CYP1A1* polymorphisms in NSCLC (non-small cell lung cancer) patients (11).

CYP1A1 or *GSTM1* polymorphisms and *p53* mutations of lung cancer in our study

During our investigations in lung cancer, one hundred twenty-three NSCLC patients, who underwent consecutive surgical resection in UOEH between 1990 and 1994, were examined for the polymorphisms in *CYP1A1* and *GSTM1* genes (12). We also examined 622 healthy individuals as controls. They lived in the northern Kyushyu region in Japan. Their lesions were pathologically conformed as follow: 71 cases were adenocarcinoma and 52 cases were squamous cell carcinoma. The incidence of *Val/Val* (homozygous of *CYP1A1**2C) and *GSTM1*; –/– were 7/123 (5.7%) and 70/123 (56.9%) in NSCLC patients, compared with 35/622 (5.6%) and 319/622 (51.3%) in healthy controls, respectively.

Table 2 Relative risk estimation of genotypes of *CYP1A1* and *GSTM1* for squamous cell carcinoma

	<i>CYP1A1 (HincII)</i>			<i>GSTM1</i>		
	<i>Ile/Ile</i>	<i>Ile/Val</i>	<i>Val/Val</i>	+/+&+/-	-/-	
n	26	21	5	22	30	
RR	1.0	1.47	2.80	1.0	1.30	
p		0.21	0.15		0.38	
95%CI		0.81–2.67	0.77–5.65		0.73–2.29	
<i>CYP1A1 (HincII)</i>						
	<i>Ile/Ile</i>	<i>Ile/Val</i>	<i>Val/Val</i>			
	<i>GSTM1</i>	<i>GSTM1</i>	<i>GSTM1</i>	<i>GSTM1</i>		
	+/+&+/-	-/-	+/+&+/-	-/-	+/+&+/-	-/-
n	10	16	10	11	2	3
RR	1.0	1.62	1.79	2.10	3.21	2.43
p		0.25	0.21	0.10	0.15	0.2
95%CI		0.72–3.64	0.72–4.43	0.87–5.09	0.67–14.46	0.63–9.49

RR; relative risk CI; confidence interval.

When the analysis was limited to squamous cell carcinoma, the incidence of *Val/Val* and *GSTM1*; –/– were 5/52 (9.6%) and 30/52 (57.7%), respectively.

The relative risks of *Ile/Val* (heterozygous of *CYP1A1**2C) or *Val/Val* in relation to *Ile/Ile* (wild-type) were 1.29 (95% confidence interval; 0.86–1.93) and 1.12 (95% CI; 0.48–2.61) in NSCLC, respectively. The relative risk of *GSTM1* –/– in relation to *GSTM1* +/+&+/- was 1.26 (95% CI; 0.85–1.85). Table 2 shows the estimation of relative risk associated with *CYP1A1* or *GSTM1* polymorphisms for squamous cell carcinoma. The relative risk of *Ile/Val* or *Val/Val* type was 1.47 and 2.08, respectively. The relative risk of *GSTM1* –/– in relation to *GSTM1* +/+&+/- was 1.30. The relative risk of combined *Val/Val* and *GSTM1* +/+&+/- was 3.21 (95% CI; 0.67–14.46), and the relative risk of combined *Val/Val* and *GSTM1* –/– was 2.43 (95% CI; 0.63–9.49) in relation to combined *Ile/Ile* and *GSTM1* +/+&+/- in squamous cell carcinoma.

The mutations in the *p53* gene are one of the most common genetic alternations found in human tumors including lung cancer. The incidence of *p53* mutations is related to the smoking index of the patients (13). The most common pattern of base change is G to T transition in lung cancer as well as in experimental animal tumors induced by BaP (14), although G to A is the major transition in colon cancer in which BaP plays a minor role (15). The *p53* mutations were common in squamous cell carcinoma (16) and also present in 43/123 (35.0%) of NSCLC patients. Table 3 shows the incidences of *p53* gene mutations accompanied by polymorphisms of *CYP1A1* and *GSTM1*. Among the NSCLC patients, the incidences of *p53* gene mutations in *Val/Val*, *C* type (*MspI*/*MspI*), and *GSTM1* –/– were 42.9%, 33.3%, and 34.3%, respectively. In squamous cell carcinoma patients, the incidences of *p53* mutations in *Val/Val* (60.0%), *C* type (50.0%) and *GSTM1* –/– (46.7%) tended to be higher than those of *Ile/Ile*&*Ile/Val* (40.4%), *A&B* type (40.5%), and *GSTM1* +/+&+/- (36.4%) and those in adenocarcinoma patients.

CYP1A1 polymorphism appears to be associated with a shortened survival in patients with non-small cell lung cancer (NSCLC)

CYP1A1 is responsible for the metabolic activation of BaP in

Table 3 Incidences of *p53* gene mutations accompanied by polymorphisms of *CYP1A1* and *GSTM1* in non-small cell lung cancer (cases)

	<i>CYP1A1</i> (<i>HincII</i>)		<i>CYP1A1</i> (<i>MspI</i>)		<i>GSTM1</i>	
	<i>Ile/Ile&Ile/Val/-</i>	<i>Val/Val</i>	<i>A&B</i>	<i>C</i>	<i>+/+&+/-</i>	<i>-/-</i>
Adenocarcinoma (n)	30.4 (69)	0.0 (2)	31.7 (60)	18.2 (11)	35.5 (31)	25.0 (40)
Squamous cell carcinoma (n)	40.4 (47)	60.0 (5)	40.5 (42)	50.0 (10)	36.4 (22)	46.7 (30)
Total	34.5 (116)	42.9 (7)	35.3 (102)	33.3 (21)	35.9 (53)	34.3 (70)

cigarette smoke. In the studies of molecular epidemiology, high susceptibility to smoking-related lung cancer has been associated with the *MspI* polymorphism of the *CYP1A1* gene. Individuals with a susceptible *CYP1A1* genotype have been found to be at significantly high risk when the genotype is combined with *GSTM1* *-/-* genotype. In our laboratory, we investigated the relations between germ line polymorphisms of these genes and clinical characteristics or survival rates in 232 patients with NSCLC. Statistical analyses demonstrated a significant association ($P<0.05$) of the *MspI* polymorphism (*MspI*) of the *CYP1A1* gene with histological type, performance status (general conditions of patients), and the extent of the primary tumor (T-factor). The *GSTM1* polymorphism was also significantly associated with performance status, the extent of regional lymph node metastasis (N-factor), and the extent of distant metastasis (M-factor). NSCLC patients with at least one susceptible allele of *MspI* in the *CYP1A1* gene (heterozygous genotype *B* or a rare homozygous genotype *C*; $n=131$) were associated with a shortened survival. (genotype *A*; $n=101$; $P=0.005$ by log-rank test). The median survival time (MST) of NSCLC patients with at least one susceptible allele was 24.2 months, although that of patients with nonsusceptible homozygous alleles was 65.2 months. Smokers with susceptible genotypes ($n=104$) were markedly associated with a shortened survival, $MST=18.2$ months, compared with 69.2 months in genotype *A* patients ($n=76$; $P=0.024$). A similar shortened survival depending on the *MspI* genotypes was not found among nonsmokers. The *MspI*-dependent shortness in survival was also observed within patients at an advanced stage of disease ($P=0.010$), but not in those at an early stage of disease ($P=0.382$). Patients with the *MspI* genotype had markedly shortened survival when the genotype was combined with a deficient genotype *GSTM1* *-/-* genotype ($P=0.017$; degree of freedom=3). Multivariate analysis by the Cox proportional hazards model also revealed that the *MspI* genotype of *CYP1A1* was an independent prognostic factor in patients at a nonresectable advanced stage of NSCLC ($P=0.005$; hazard ratio=1.98; 95% confidence interval, 1.24-3.17). When NSCLC patients are separated on the basis of *CYP1A1* polymorphisms, NSCLC patients with *Val/Val* (**2C/*2C*) may also have a shortened survival than the patients with *Ile/Ile&Ile/Val* (Fig. 2) although the number of *Val/Val* patients are not yet sufficient for conclusion. Arylhydrocarbons (AH), such as BaP, are procarcinogens associated with lung cancer and also induce phase I and phase II enzymes. Therefore, the *Val* mutant P450 enzyme may delay the activation of AH but may also accumulate AH in cells, which results in increased levels of phase I and phase II enzymes

Fig. 2 Survival curves of NSCLC patients in relation to *CYP1A1* polymorphism.

via the induction mediated by AH receptor. Therefore, the *Val/Val* genotype decreases the activation of AH mediated by *CYP1A1* but may increase the expression level of other P450s that convert AH to active carcinogens (DNA-binding metabolites), resulting in an increased production of activated carcinogens. Therefore, when the *CYP1A1* polymorphism is accompanied by *GSTM1* *-/-* genotype, the cellular levels of AH and activated AH may be increased but the detoxification may be delayed. This delay could be associated with the increased incidence of *p53* gene mutations in lung cancer. Since *p53* mutations in NSCLC have significant negative prognostic values (17-20), *CYP1A1* polymorphisms may be associated with clinical prognostic parameters including *p53* mutations.

In summary, carcinogenesis involves complex multiple processes that may be triggered by the activation of chemical carcinogens. Most sporadic cancers occur due to the exposure of carcinogens and are mediated by inherited susceptibilities for carcinogen metabolisms that are altered by the variations in chromosomal DNA sequence. Although social and ethical problems are present in the use of genetic information, individual profiles of putative susceptibility genes will be of great help for people with susceptible genes to reduce the cancer risk by carcinogen exposure.

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References

- (1) Kinzler KW, Vogelstein B. Gatekeepers and caretakers. *Nature* 1997; 386: 761–763.
- (2) Vineis P, Porta M. Casual thinking, biomarkers, and mechanisms of carcinogenesis. *J. Clin. Epidemiol.* 1996; 49: 951–956.
- (3) Harris CC. Interindividual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair. *Carcinogenesis* 1989; 10: 1563–1566.
- (4) Bouchardy C, Benhamou S, Jourenkova N, Dayer P, Hirvonen A. Metabolic genetic polymorphisms and susceptibility to lung cancer. *Lung Cancer* 2001; 32: 109–112.
- (5) Lai C, Shields PG. The role of interindividual variation in human carcinogenesis. *J. Nutr.* 1999; 129: 552s–555s.
- (6) Hayashi S-I, Watanabe J, Nakachi K, Kawajiri K. Genetic linkage of lung cancer-associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. *J. Biochem.* 1991; 110: 407–411.
- (7) Eaton DL, Bammler TK. Concise review of the glutathione S-transferases and their significance to toxicology. *Toxicol. Sci.* 1999; 49: 156–164.
- (8) Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in *Escherichia coli* of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *J. Biol. Chem.* 1997; 272: 10004–10012.
- (9) Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 1998; 19: 275–280.
- (10) Nakachi K, Imai K, Hayashi S-I, Kawajiri K. Polymorphisms of the *CYP1A1* and Glutathione S-Transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res.* 1993; 53: 2994–2999.
- (11) d'Errico A, Malats N, Vineis P, Boffetta P. Chapter 23. Review of studies of selected metabolic polymorphisms and cancer. In: Vineis P, Malats N, Lang M, d'Errico A, Caporaso N, Cuzick J and Boffetta P (eds.) *Metabolic polymorphisms and susceptibility to cancer*. Lyon; International Agency for Research on Cancer, 1999: 323–393.
- (12) Oyama T, Kawamoto T, Mizoue T, Nishida K, Osaki T, Sugio K, Yasumoto K, Mitsudomi T. p53 mutations of lung cancer are not significantly affected by *CYP1A1* or *GSTM1* polymorphisms. *Int. J. Oncol.* 1997; 11: 305–309.
- (13) Suzuki H, Takashi T, Kuroishi T, Suyama M, Ariyoshi Y, Takahashi T, Ueda R. p53 mutations in non-small cell lung cancer in Japan: association between mutations and smoking. *Cancer Res.* 1992; 52: 734–736.
- (14) Strauss BS. The origine of point mutations in human tumor cells. *Cancer Res.* 1992; 52: 242–253.
- (15) Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JKV, Hamilton S, Vogelstein B. p53 mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res.* 1990; 50: 7717–7722.
- (16) 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.
- (17) Korkolopoulou P, Oates J, Crocker J, Edwards C. p53 expression in oat and non-oat small cell lung carcinomas: correlations with proliferating cell nuclear antigen. *J. Clin. Pathol.* 1993; 46: 1093–1096.
- (18) Ebina M, Steinberg SM, Mulshine JL, Linnolia RI. Relationship of p53 overexpression and up-regulation of proliferating cell nuclear antigen with the clinical course of non-small cell lung cancer. *Cancer Res.* 1994; 54: 2496–2503.
- (19) Tormanen U, Eerola AK, Rainio P, Vahakangas K, Soini Y, Sormunen R, Bloigue R, Lehto VP, Paakko P. Enhanced apoptosis predicts shortened survival in non-small cell lung carcinoma. *Cancer Res.* 1995; 55: 5595–5602.
- (20) Esposito V, Baldi A, De Luca A, Micheli P, Mazzarella G, Baldi F, Caputi M, Giordano A. Prognostic value of p53 in non-small cell lung cancer: relationship with proliferating cell nuclear antigen and cigarette smoking. *Hum. Pathol.* 1997; 28: 233–237.