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Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P450IA1 gene

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A good correlation was observed between enhanced lung cancer risk and restriction fragment length polymorphisms (RFLPs) of the P450IA1 gene with the restriction enzyme *Msp*I. Genotype frequencies of 0.49 for the predominant homozygote, 0.40 for the heterozygote, and 0.11 for the homozygous rare allele were observed in a healthy population. Among lung cancer patients, the frequency of homozygous rare allele of P450IA1 gene was found to be about 3-fold higher than that among healthy population, and this difference was statistically significant. This is the first report to identify the genetically high risk individuals to lung cancer at the gene level.

Cytochrome P450IA1 gene; Restriction fragment length polymorphism; Lung cancer

1. INTRODUCTION

Microsomal mixed-function oxidations are essential in the metabolism of drugs, steroids and chemical carcinogens [1,2]. Genetic variation among individuals in metabolism have been reported [3,4], and variations in the oxidative activation of chemical carcinogens might well explain differences in susceptibility of individuals to chemical carcinogens [2]. The metabolism of chemical carcinogens involves a variety of isozymes of cytochrome P450, and differences in their genotypes or phenotypes may be a main factor responsible for differences among individuals in susceptibility to carcinogens.

Lung cancer is one of the most common cancers in all countries, and squamous cell carcinoma is known to be caused by cigarette smoking. Initiation of the carcinoma requires metabolic activition of the procarcinogens such as benzo(a)pyrene to the ultimate form by cytochrome P450IA1 (so-called P-450c or P_1-450) [5]. Accumulated evidence [6,7] suggests that increased risk of smoking-induced bronchogenic carcinoma is associated with high activity of aryl-hydrocarbon hydroxylase (AHH), an inducibility phenotype of P450IA1.

Restriction fragment length polymorphisms (RFLPs) of human P450IA1 gene have been detected [8], although no association with cancer incidence has been reported. In this paper, we report that *Msp*I polymorphisms associated with genetically determined differences among individuals in their susceptibility to lung cancer.

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2. EXPERIMENTAL

Blood samples (7–15 ml) were obtained from individuals, and DNA was isolated from peripheral lymphocytes. Human lymphocyte DNA (8 μg) was digested completely with restriction endonuclease *Msp*I (Takara, 60 units) for 3 h at 37°C and the products were subjected to electrophoresis in 0.8% agarose for Southern blot analysis [9]. The DNA fragments were transferred to a nitrocellulose membrane (Schleicher and Schüll), incubated with nick-translated DNA probes labeled with ^{32}P-dCTP (Amersham).

The DNA fragment including *Msp*I site at 3047 was amplified from the individual (lane 1 in Fig. 2a) by PCR method [10]. Used were the primers of 21 bases from 2901 of the coding and 3197 of the reverse complement sequence of P450IA1 gene [11].

3. RESULTS AND DISCUSSION

When lymphocyte DNAs were digested with *Msp*I and hybridized with the *Xba*I-*Eco*RI fragment (probe 1 in Fig. 1a), clear RFLPs of the P450IA1 gene were observed among individuals. Fig. 2a shows representative results on DNA polymorphisms observed in unrelated individuals by Southern blot analysis. The RFLPs detected with *Msp*I can be classified into 3 types: type A is characterized by 2.7, 2.3, and 0.8 kb fragments (lanes 3, 4, 5 and 8 in Fig. 2a), type B by 2.7, 2.3, 1.9 and 0.8 kb (lanes 6, 7, 9 and 10), and type C by 2.7, 1.9 and 0.8 kb (lane 11). The lengths of these bands agreed well with those determined by sequence analysis (Fig. 1b) [11]. Our results by Southern blot (Fig. 2a) and sequence (Fig. 1b) analyses showed that

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Fig. 1. Structural organization and restriction cleavage sites for *Msp*I of the P450IA1 gene. The restriction map and organization of the P450IA1 gene cloned into λhP-450mc-1 [11] are shown in (a). The exons are shown by closed boxes and restriction sites are marked by X for *Xba*I and E for *Eco*RI. Sites of *Msp*I were determined from sequence analysis [11] and shown in (b) by vertical lines. The two sites indicated by ▲ and ★ are origins of polymorphisms as described in the text. The probes used are shown by 1 and 2 in (a). The fragment lengths corresponding to the observed bands are shown in (b).

the 3 polymorphisms originated from presence or absence of one *Msp*I site (▲ in Fig. 1b) at the 3'-end. We then carried out genomic blot analysis using the *Eco*RI-*Eco*RI fragment (probe 2 in Fig. 1a) and found

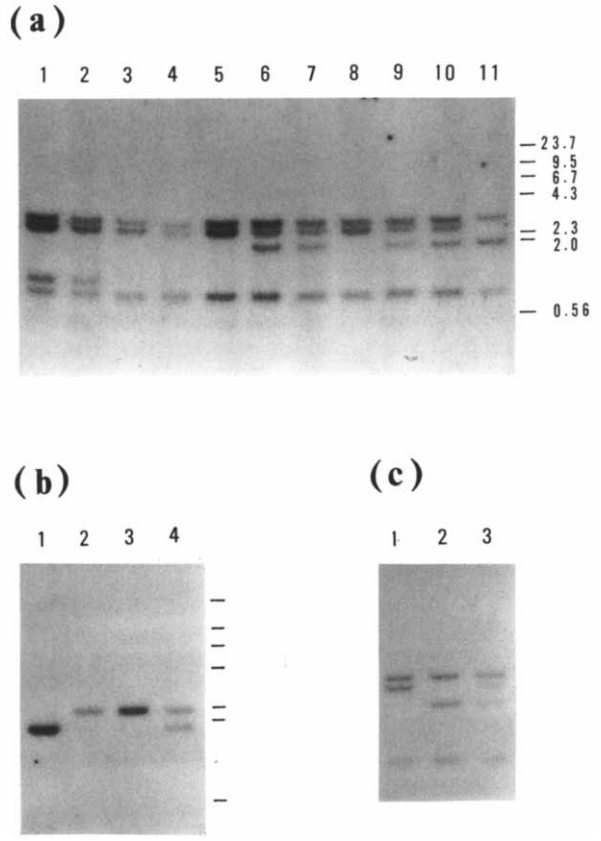

Fig. 2. DNA polymorphisms of the P450IA1 gene by *Msp*I. (a) Probe 1 (*Xba*I to *Eco*RI in Fig. 1a) was used for unrelated individuals. λDNA digested with *Hin*dIII was used as a size marker. (b) Probe 2 (*Eco*RI to *Eco*RI in Fig. 1a) was used. Lanes 1–4 were obtained from the same DNA as lanes 11, 1, 3 and 9, respectively, in (a). (c) Polymorphisms of related individuals examined with probe 1. Lanes 1, 2 and 3 show paternal, maternal DNA and that of their child, respectively.

3 types of *Msp*I polymorphisms (Fig. 2b). We also obtained the segregation profile of polymorphic *Msp*I fragments by family pedigree analysis (Fig. 2c). Accordingly, we concluded that the 3 polymorphisms consisted of two homozygous (types A and C) and one heterozygous states (type B) at a single locus.

In a few subjects, we observed an additional band of 1.0 kb (lanes 1 and 2 in Fig. 2a). The gene sequence [11] suggested that this band could be produced by a point mutation or methylation of the *Msp*I site in the 2nd exon at 3047 (★ in Fig. 1b) from the transcription-initiation site [11]. We concluded, however, that it was not ascribable to a point mutation of the *Msp*I site, but to specific methylation of the site, because agarose gel electrophoresis of the DNA amplified by PCR with *Msp*I digestion showed a complete digestion pattern (Fig. 3).

We then determined the frequency of each type of *Msp*I polymorphism in the general population. We isolated lymphocyte DNA from a cohort of 1500 persons of over 40 years old and analyzed the DNA polymorphisms of 104 randomly selected subjects as healthy controls (Table I). Types A (m1/m1), B (m1/m2), and C (m2/m2) were found respectively in 51, 42 and 11 of these healthy controls. This result gave a good fit to the Hardy-Weinberg equilibrium with a gene frequency of 0.69 for m1 and 0.31 for m2.

In Table I, the frequencies of genotypes among lung cancer patients were different from those among healthy controls with statistical significance of $P < 0.05$ ($\chi^2 = 6.182$ with d.f. = 2). In addition, the comparison of genotypes A+B or A with C in frequencies also showed significant difference with $P < 0.05$, allowing us to estimate the risk elevation (odds ratio) of C type to be 2.6 or 3.1 compared with A+B or A types. Among the histological types, the squamous cell carcinoma gave remarkable deviation of frequencies from

Fig. 3. *Msp*I digestion of amplified DNA indicating specific methylation of the *Msp*I site at 3047 of the P450AI1 gene. Lanes 3 and 2 were digested with or without *Msp*I, and lane 1 is a size marker of SV-40 DNA digested with *Hin*dIII.

Table I

Distribution of the 3 types of P450IA1 gene among lung cancer patients and healthy controls

Population	P450IA1 genotye			Total
	A(m1/m1)	B(m1/m2)	C(m2/m2)	
Healthy controls (H)	51(49.0)	42(40.4)	11(10.6)	104(100%)
Lung cancer (L)	24(35.3)	28(41.2)	16(23.5)	68(100)
Squamous cell carcinoma (S)	7(30.4)	9(39.1)	7(30.4)	23(100)
Small cell carcinoma	8(44.5)	6(33.3)	4(22.2)	18(100)
Large cell carcinoma	1(16.7)	4(66.6)	1(16.7)	6(100)
Adenocarcinoma	8(38.1)	9(42.9)	4(19.0)	21(100)
Odds ratio				
Between H and L	1.0	1.417	3.091	
	1.0		2.601	
Between H and S	1.0	1.561	4.636	
	1.0		3.699	

Risk of C type relative to A or A + B type is also shown as odds ratio

the healthy controls ($\chi^2 = 6.675$ with d.f. = 2). The risk of C type was calculated to be about 5-fold for this cell type of lung cancer comparing with A type. We conclude that the individuals with the homozygote rare allele of *MspI* polymorphisms for the P450IA1 gene are at 3-–5-fold higher risk of lung cancer, especially squamous cell carcinoma, than those with other genotypes. This risk elevation of C type was specifically observed for lung cancer because the frequencies of C type among other two cancers were identical to those of healthy controls (5 out of 39 stomach cancer patients and 3 out of 31 breast cancer patients).

The frequency of 3 genotypes for P450IA1 among healthy controls showed an excellent agreement with the frequencies of tri-modal AHH inducibility observed in general population [12,13]. Moreover, the proportion of patients with the homozygote rare allele (type C) among those with squamous cell carcinoma was the same as that of patients with the high AHH phenotype among those with bronchogenic carcinoma [6]. The difference of P450IA1 genotypes in risk of lung cancer may be explained by the tri-modal AHH inducibility. To examine this, we are now comparing the structures and expressions of the genotypes of P450IA1.

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