l-myc Polymorphism in Gastric Cancer, Lung Cancer, and Hepatocellular Carcinoma

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Purpose: This study was performed to see if a particular polymorphism in the *l*-myc, a nuclear oncogene at the 1p32 locus, might be associated with greater risk of gastric cancer, lung cancer and hepatocellular carcinomas (HCC) in Korean patients.

Materials and Methods: Genomic DNA, derived from patients diagnosed with gastric cancer (n=57), lung cancer (n=39), HCC (n=35) and healthy individuals (n=176), was examined. The *l*-myc polymorphism under study was visualized by PCR followed by *Eco*RI digestion.

Results: There was no significant difference in the

distribution of the *l*-myc polymorphism genotypes and allele frequencies between the cancer patients and the controls.

Conclusion: The *l*-myc polymorphism does not appear to be indicative of elevated risk of cancers of the stomach, lung and HCC. (Cancer Research and Treatment 2002;34: 436-438)

Key Words: Gastric neoplasm, Lung neoplasm, Hepatocellular carcinoma, *l*-myc polymorphism

INTRODUCTION

Gastric cancer, lung cancer, and hepatocellular carcinomas (HCC) are major causes of cancer-related deaths in Koreans. Previous studies have tried to identify possible genetic markers for the assessment of individual risk to gastric cancer, lung cancer and HCC. Several epidemiological, pharmacogenomic and genetic studies have postulated the possibility of an inherited predisposition to gastric cancer, lung cancer and HCC (1~6). Activating proto-oncogenes, and turning them into oncogenes, is an important mechanism of the initiation and progression of tumorigenesis (7). Previous studies have suggested that allelic variation of proto-oncogenes, such as *l*-myc, a nuclear oncogene at the 1p32 locus, might predispose individuals towards tumor development (8~10), implying that such variant alleles may serve as markers for genetic susceptibility to cancer.

Recently, polymorphisms of the *l*-myc gene have been investigated in various types of cancer (10~19). However, the results from these investigations have not always been

consistent (9,12). Moreover, these studies have not investigated the potential utility of the *l*-myc polymorphism as a genetic marker in the Korean population.

In this study, we have examined if allelic variants of the *l*-myc gene are associated with an increased risk for gastric cancer, lung cancer and HCC in the Korean population.

MATERIALS AND METHODS

1) Patient selection

Our sample population comprised of 57 patients diagnosed with gastric cancer, 39 with lung cancer, 35 with HCC, and 176 normal individuals, all of Korean ethnicity. The average age of patients was 42 years, ranging from 16 to 79 years of age; lung cancer: 66, stomach cancer: 62, HCC: 56. The control group ages were adjusted according to relate to those of the patients. Informed consents were gained from all the lung cancer, hepatocellular carcinoma and stomach cancer cases. Blood samples were obtained without any particular exclusion criteria. All the control patients had to have no previous history of cancer, and were in sufficiently good condition to provide a blood sample.

2) PCR-RFLP analysis of *l*-myc

Genomic DNA was extracted from peripheral blood leukocytes according to routine procedures. The polymorphism in the 2nd intron of the *l*-myc gene was amplified using primers that flank the polymorphic *Eco*R I site, as described below (9):

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10 pmol of each primer (5'-AGTTCACCTACAGGCCACAT-3' and 5'-TGCATATCAGGAAGCTTGAG-3'), 2.5 mM of dNTP, 2 units of *Taq* polymerase (Takara, Shiga, Japan) and 3 µl of 10X PCR buffer were mixed together, and the restriction volume was brought up to 30 µl with ddH₂O. The PCR products were digested with *Eco*RI (Roche, Mannheim, Germany) at 37°C overnight, which was followed by electrophoresis on a 2.5% agarose gel containing ethidium bromide for visualization.

3) Statistical analysis

Statistical analysis of differences of the genotypic and allelic frequencies of the polymorphic forms of the *l-myc* gene, between the patients and the control subjects, was determined using the χ^2 test. $P < 0.05$ was considered to be indicative of statistical significance.

RESULTS

The characteristics of the patients and controls are shown in Table 1. All the cancer patients had biopsy proven malignancies, and lung cancer pathologic subtypes were shown (NSCLC; 31 cases, SCLC; 8 cases).

The bands corresponding to the polymorphic fragments of

Table 1. Characteristics of cancer patients and control subjects

		Case	Control
Total (n)		131	176
Stomach cancer (adenocarcinoma)		57	
Lung cancer	NSCLC	31	
	SCLC	8	
Hepatocellular carcinoma		35	
Gender	Male	108	90
	Female	23	86
Age	< 40	2	96
	40 ~ 49	16	69
	50 ~ 59	36	10
	60 ~ 69	44	1
	≥ 70	33	0

each *l-myc* *Eco*RI genotype read as follow. Two alleles were observed upon *Eco*RI digestion: the 'L' allele, corresponding to the 267 bp fragment, and lacking the *Eco*RI site, and the 'S' allele, corresponding to the 142 bp and 125 bp fragments, produced from the cleavage at the *Eco*RI site. The alleles were made up of three genotypes, LL (the 267 bp fragment only), LS (three fragments, the 267 bp, 142 bp and 125 bp fragments) and SS (two segments, the 142 bp and 125 bp fragments) (Fig. 1).

The LL : LS : SS ratios (ratio of the genotypes) were 22 : 26 : 9, 17 : 20 : 2 and 17 : 16 : 2 in the gastric cancer, lung cancer and HCC patient groups, respectively (Table 2). There were no significant differences in the *l-myc* genotype distribution and allele frequencies between the cancer patients and the healthy individuals. To determine any possible association between the *l-myc* genotype and the risk of histological types of lung cancer, cases were divided into two groups according to their pathological classification. No differences were seen in the *l-myc* genotype distribution between the two groups, but because the sample size was small, proper risk determination was insufficient.

DISCUSSION

Recently, studies for various cancer risk and polymorphisms of candidate genes have been reported, for which epidemiological and clinicopathological associations with genetic susceptibility to cancer were discussed (20 ~ 22).

In the present report, a genotypic association study was performed to investigate a possible relationship between the *l-myc* *Eco*RI polymorphism and susceptibilities to gastric cancer, lung cancer and HCC.

Fig. 1. Representative photos of genetic polymorphism analysis representing the three patterns of genotypes. Homozygote LL allele (Lane 3, 5), Heterozygote LS allele (Lane 2, 4), Homozygote SS allele (Lane 1) for *L-myc* *Eco*RI RFLP.

Table 2. Distribution of polymorphism of *l-myc* among gastric cancer, lung cancer, and hepatocellular carcinoma (HCC) patients and controls

Group	<i>l-myc</i> genotype					<i>l-myc</i> allele			
	LL	LS	SS	X ²	P	L	S	X ²	P
Gastric cancer	22 (0.39)	26 (0.46)	9 (0.15)	0.90	0.64	70	44	0.08	0.78
Lung cancer	17 (0.44)	20 (0.51)	2 (0.05)	2.84	0.24	54	24	2.33	0.13
HCC	17 (0.49)	16 (0.46)	2 (0.05)	3.60	0.16	50	20	3.26	0.07
Controls	59 (0.33)	93 (0.53)	24 (0.14)			211	141		

Several previous studies have examined the frequencies of *l-myc* genotypes in various types of human cancer, such as gastric (14,19), lung (13,16), colorectal (12,17), HCC (10), oral (15), breast (19), renal (11) and bone or soft tissue sarcomas (18), for possible association between various polymorphisms and cancer susceptibility.

It remains controversial as to whether the *l-myc* polymorphism may affect the susceptibility to tumors. Kato et al (18) reported an association between the SS genotype and susceptibility to bone and soft tissue sarcomas in male patients, but no such association was found with lymphoma, leukemia or breast cancer. Taylor et al (10) reported a protective effect of the SS genotype in HCC.

In the present study, there were no significant differences in the genotypic and allele frequencies of the *l-myc* gene between the cancer patients and the controls.

CONCLUSIONS

The *l-myc* *EcoRI* polymorphism may not be associated with increased susceptibility to gastric cancer, lung cancer or HCC.

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