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Association of the Human Uteroglobin Gene Polymorphism with Primary Lung Cancer

Purpose: Human uteroglobin (hUG) is a cytokine-like multifunctional protein that possesses potent immunomodulatory and anti-tumor activity. And, the G38A polymorphism of uteroglobin exon 1 has been associated with the development of immunoglobulin A nephropathy, systemic lupus erythematosus and bronchial asthma. In addition, the 38AA genotype has been related to lower serum levels of uteroglobin than the GG or GA genotypes. Although the hUG gene is one of the candidate tumor suppressors in lung cancer, the uteroglobin gene polymorphism has not been reported upon in lung cancer. Therefore, we studied the frequencies of the G38A polymorphism of the hUG gene in patients with lung cancer and control subjects to investigate its relation with lung cancer. **Materials and Methods:** A matched case control design study was adopted to investigate the possibility of an association between primary lung cancer and the G38A polymorphism. To exclude the possible influence of tobacco smoke exposure, or of age or gender on the development of lung cancer, these factors were matched between the 60 patients and the 60 controls. Genotypes were determined by polymerase chain reaction followed by restriction fragments length polymorphism analysis for the hUG gene. **Results:** The frequency of the 38A allele in patients was 0.55, which was significantly different from its frequency of 0.37 in controls (p=0.007). Moreover, the frequency of the 38AA genotype was significantly higher in patients (35%) than in controls (15%) (p=0.01). Furthermore, two patients previously diagnosed as having prostate cancer were all genotyped as 38AA. **Conclusion:** The G38A polymorphism of the hUG gene is associated with the development of primary lung cancer in Koreans. **(J Lung Cancer 2006;5(1):30－34)**

Key Words: Lung cancer, Uteroglobin, CC10, Polymorphism, Prostate cancer

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INTRODUCTION

Blastokinin(1), also called uteroglobin (UG)(2), is a steroid-inducible, multifunctional protein secreted by the mucosal epithelia of many organs, including the uterus, lung, mammary gland, and the prostate in virtually all mammals(3). It is a dimer with a molecular mass of 16 kD, but due to its anomalous electrophoretic mobility, it migrates as a 10 kD protein in polyacrylamide gel electrophoresis. Thus UG is also known as a Clara cell 10 kD protein (CC10)(4). Numerous names have been ascribed to this protein, primarily based on the tissue or body fluid in which it was first detected(1,2,5～7). UG is the most common protein in the bronchoalveolar lavage fluid (BALF) of healthy non-smokers, averaging 7% of the BALF total protein(8), and the immunomodulatory activity of UG has been well documented(9,10).

The human UG (hUG) gene, mapped to chromosome 11q 12.2～13.1, consists of three exons and two introns and is structurally conserved during evolution(11). Interestingly, rearrangements or deletions of this region of chromosome 11 have been correlated with human cancers(12～14). Recent reports indicate that though the hUG gene is constitutively expressed at a high level in the mucosal epithelia of many organs (e.g., the lung, mammary gland, uterus, and prostate)(3), the hUG expression is either drastically suppressed or totally lacking in adenocarcinoma tissues and the cell lines derived from ade-

nocarcinomas of the lung(15,16).

Recently, a single nucleotide polymorphism at the 38th nucleotide (G38A) downstream of the transcription initiation site of hUG gene within the noncoding region of exon 1 has been reported in immunoglobulin A (IgA) nephropathy, systemic lupus erythematosus (SLE) and bronchial asthma(17~20). Moreover, investigations have revealed that subjects with the 38AA genotype of this gene have lower serum levels of UG than those with 38GG or 38GA genotype(20,21).

Although the hUG gene is a candidate tumor suppressor in lung cancer, hUG gene polymorphisms have not been previously reported in patients with primary lung cancer. Therefore, the aim of this study was to test association between the G38A polymorphism of the hUG gene and primary lung cancer.

METHODS

1) Study design and study subjects

A matched case control design study was adopted to examine the possible association between the hUG gene G38A polymorphism and primary lung cancer. According to a previous report, the frequency of homozygotes of G38A (38AA) is 0.22 in Koreans(18). We used Lehr's formula(22) to calculate the sample size required for a power of 90% and a two-sided significance level of 0.05. Using this formula, the required sample size in each group was determined to be 48 to detect a statistical difference in 38AA frequencies of 0.20 between patients and controls. Thus, 60 patients were recruited from the Department of Internal Medicine, Seoul National University Hospital. A diagnosis of lung cancer was based on the results of cytology or biopsy, which were obtained by bronchoscopy, percutaneous needle aspiration, or surgery. Twenty-two of the 60 patients had been previously diagnosed as having chronic obstructive pulmonary disease (COPD). Sixty control subjects were also recruited form the Health Promotion Center of Seoul National University Hospital; 23 of subjects had COPD and 37 were healthy. To exclude the possible influence of tobacco smoke exposure, or of age or gender on the development of lung cancer, these factors were matched in the two groups (Table 1).

Table 1. Characteristics of Study Subjects

	Patients	Controls	p value
Age (years)	61.9±1.4	61.9±1.4	>0.05
Male/female	48/12	48/12	>0.05
Smoker/non-smoker	45/15	45/15	>0.05
Pack-years	28.7±3.4	27.6±3.3	>0.05
FVC (% predicted)	81.4±2.8	83.8±2.8	>0.05
FEV1 (% predicted)	77.3±3.2	83.9±4.6	>0.05
FEV1/FVC (%)	69.1±1.6	71.1±2.6	>0.05
COPD, no.	22	23	>0.05

FVC: forced vital capacity, FEV1: forced expiratory volume in 1 second

2) Extraction of genomic DNA and genotype determination

Genomic deoxyribonucleic acid (DNA) was extracted from whole blood by standard methods using a commercially available kit (QIAamp^R DNA purification kit, Qiagen, USA). Thereafter, the G to A polymorphism at the 38th nucleotide downstream from the transcription initiation site within exon 1 of hUG gene was determined. The nucleotide sequence of the hUG gene was obtained from the GenBank (accession number; X59875) and used to design appropriate primers to amplify of exon 1 by polymerase chain reaction (PCR): sense: 5'-GGA ATA TTT ACC TAT CCC ACC AAG C-3', antisense: 5'-CCT GAG AGT TCC TAA GTC C-3'. For the PCR amplification of exon 1,100 ng of genomic DNA was used as template in a 25 μl reaction mixture, which contained the following reagents: 3 pmol of each primer (Bioneer, Korea), 1.25 U of recombinant Taq DNA polymerase (Takara Shuzo Co., Kyoto, Japan), X10 reaction buffer (100 mmol/l Tris-HCl, pH 8.3 at 25°C, 500 mmol/l KCl), 1.5 mmol/l $MgCl_2$, and 200 μmol/l of each deoxynucleotide triphosphate (Takara Shuzo Co., Kyoto, Japan). Following an initial denaturation at 94°C for 5 minutes, the reactions were cycled 35 times through a temperature profile of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. A final extension was performed at 72°C for 10 minutes. The products of the reaction were visualized by electrophoresis in a 1.2% agarose gel containing ethidium bromide (1.5 μg/ml). PCR yielded fragments containing exonic sequences of 302 bp. Study subjects were genotyped using Sau96I endonuclease (Promega, Korea), which produced 130-bp and 172-bp fragments for the 38G allele, whereas the 302-bp 38A alleles, which lacking the *Sau*96I site, was not cut (Fig. 1). Digested DNA samples were examined following electrophoresis on a 1.8% agarose gel containing ethidium bromide

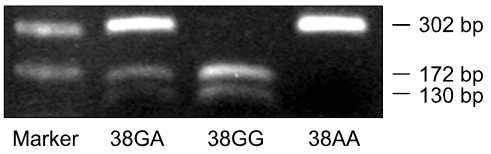

Fig. 1. Polymerase chain reaction-restriction fragment length polymorphism analysis of the UG genotypes.

(1.5 μg/ml). DNA was visualized using a single intensity transilluminator (300 nm) and photographed using the Gel-Doc system (Bio-Rad Laboratories, Hercules, CA, USA).

3) Statistical analysis

All statistical analyses were performed using SPSS for Windows Release 10.0.1 (SPSS Inc., Chicago, USA). Data are presented as means±standard error of the mean. χ^2 analysis was used to detect differences in the genotype distributions of patients and controls. A p value of <0.05 was considered significant.

RESULTS

Demographic and clinical characteristics are summarized in Table 1. None of the study subjects had a past medical history of bronchial asthma, SLE, or IgA nephropathy. However, two patients with primary lung cancer had been previously diagnosed as having prostate cancer. They were all genotyped as 38AA. Otherwise, no subject reported a past medical history of malignancy.

The 38A allele frequency was found to be significantly higher in the lung cancer patients (0.55) than in the controls (0.37, odds ratio, 2.04, 95% confidence interval, 1.22 to 3.41, p=0.007) (Table 2). Moreover, the frequency of the 38AA genotype was significantly higher in patients (35%) than in controls (15%, odds ratio, 3.05, 95% confidence interval, 1.26 to 7.40, p=0.01) (Table 2).

We next compared a histological diagnosis of lung cancer, and tobacco smoke exposure in the patient group according to the 38GG/38GA or 38 AA genotypes (Table 3), and found no statistically significant differences.

DISCUSSION

In the present study, the G38A polymorphism was identified in exon 1 of the hUG gene in patients with primary lung cancer. Although the published allele was 38A, 38G was found to be present at a higher frequency than 38A in the control subjects, which is in agreement with previous findings(19~21,23). This finding suggests that the 38G allele is more likely to be the wild type. However, Kim et al. reported that the frequency of the 38A allele (0.51) is similar to that of 38G (0.49) in Koreans(18). Therefore, further study is required to determine the frequencies of 38G and 38A, in Asians.

UG is a cytokine-like multifunctional protein that possesses potent immunomodulatory activity. According to previous reports, the G38A polymorphism is related to IgA nephropathy, SLE, and bronchial asthma(17~20). Matsunaga et al. reported that the 38AA genotype is more common in patients with IgA nephropathy (24%) than in healthy controls (12%)(20). Menegatti et al. also reported that the frequency of 38A allele is higher in SLE patients (0.59) than in normal controls (0.37)(17). Furthermore, the 38 AA genotype is related to decreased serum levels of UG in patients with IgA ne-

Table 2. Genotypes and Allele Frequencies for the hUG Gene Mutation in Patients and Controls

	No. of subjects	Genotype			Allele frequency	
		38GG	38GA	38AA	38G	38A
Patients	60	15 (25)	24 (40)	21 (35)*	0.45	0.55†
Controls	60	24 (40)	27 (45)	9 (15)	0.63	0.37

Values expressed as number (percent). *$p<0.05$: significant difference in the 38AA genotype frequency between patients and controls, †$p<0.05$: significant difference in the 38A allele frequency between patients and controls

Table 3. Genotypes and Clinicopathological Characteristics in the Patient Group

	38GG or 38GA	38AA	p value
Histological diagnosis of lung cancer, no. (%)			>0.05
Non-small cell carcinoma	36 (92.3)	16 (76.2)	
Squamous cell carcinoma	18 (46.2)	5 (23.8)	
Adenocarcinoma	11 (28.2)	9 (42.9)	
Undifferentiated	7 (17.9)	2 (9.5)	
Small cell carcinoma	3 (7.7)	5 (23.8)	
Smoker/non-smoker, no.	31/8	14/7	>0.05

phropathy and bronchial asthma(20,21). These results suggests that the hUG gene polymorphism may alter UG protein expression, and therefore influence the development of IgA nephropathy or bronchial asthma.

Uteroglobin also possesses potent anti-tumor activity. In adenocarcinoma tissues and in cell lines derived from adenocarcinomas of the lung, hUG expression is either drastically suppressed or totally lacking(15,16); moreover UG suppresses extracellular matrix invasion by cancer cells(24). Although the hUG gene may be a candidate tumor suppressor in malignancies, its polymorphism has not been reported upon in malignancies.

In the present study, 38A allele was shown to be associated with a significantly higher risk of developing primary lung cancer. Furthermore, the 38AA genotype was also found to be associated with the development of lung cancer. Although we did not measure the serum levels of UG in the study subjects, decreased serum levels of UG has been reported in subjects with the 38AA genotype(20,21). To the best of our knowledge, the present study is the first to elucidate an association between a hUG gene polymorphism and primary lung cancer. However, it is not clear how this hUG gene polymorphism contributes to the development of lung cancer. There is a possibility that the polymorphism may alter UG protein expression, and thus influence lung cancer development.

Regarding the correlation between genotypes of the hUG gene and clinicopatholgical factors, no significant relationship was found. Although the diagnosis of adenocarcinoma or small cell carcinoma was more common in patients with 38AA than 38GG/38GA, this result was not statically significant.

In this study, two patients with primary lung cancer had been previously diagnosed as having prostate cancer. They were all genotyped as 38AA. This result suggests that the 38AA genotype may be related to multiple malignancies. Indeed, the associations between UG and malignancy have been reported by previous studies(12～14). Furthermore, UG expression is lacking in the tissue of prostate cancer(14), and recombinant human UG was found to inhibit the in vitro invasiveness of human metastatic prostate tumor cells(25). Further study is required to determine whether the hUG gene polymorphism contributes to multiple malignancies, such as lung cancer or prostate cancer.

In conclusion, this study identified a significant relationship between genotype differences at position 38 of exon 1 of the hUG gene and the development of lung cancer. Further study is needed to elucidate the mechanism of this phenomenon.

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