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Original article

A TERT-CLPTM1 locus polymorphism (rs401681) is associated with EGFR mutation in non-small cell lung cancer

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ABSTRACT

Telomere length is associated with lung carcinogenesis, and recent studies have focused on telomere-maintaining genes and their polymorphisms. Cancer susceptibility of the rs401681 polymorphism, located in the TERT-CLPTM1L locus, has been studied in many cancers. We examined the clinicopathological and prognostic value of rs401681 variants in lung cancer. The relationship between rs401681 variants and telomere length was analyzed in 134 non-small cell lung cancers (NSCLCs). The rs401681 polymorphism had the following genotype frequencies: C/C in 52.2% of the samples, C/T in 30.6%, and T/T in 17.2%. The T allele showed a strong correlation with EGFR mutation ($p = 0.037$). Telomeres in the tumor samples were 3.26-fold longer, on average, than telomeres in matched normal samples (SD = 0.48), and there were no differences in telomere length according to rs401681 polymorphism. Smoking was associated with telomere shortening ($p = 0.01$). Survival analysis showed no prognostic value for rs401681 polymorphisms or telomere length in NSCLC. These results suggested that the rs401681 polymorphism contributes to lung carcinogenesis only in patients harboring an EGFR mutation. However, the polymorphism was not associated with survival; therefore, further comprehensive analysis should be performed.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, and the general prognosis for lung cancer remains poor, with 5-year survival rates < 15%, despite recent advances in treatment [1]. Lung cancer can be subdivided into small cell lung carcinoma and non-small cell lung carcinoma (NSCLC). Epidermal growth factor receptor (EGFR) alterations are involved in the pathogenesis and progression of many malignancies, including lung cancer [2]. EGFR mutations are present in more than half of NSCLC cases, and are associated with poor prognosis and chemoresistance [3,4]. Moreover, the expression of EGFR appears to be dependent on histological subtype in NSCLC, being most frequently expressed in squamous cell carcinoma. The most widely recognized genomic alterations include EGFR mutations, which have been more frequently reported in Asian than in non-Asian patients [5]. However, other factors, such as clinicodemographic and molecular factors, also regulate genetic variants leading to the progression of NSCLC [6].

Telomeres form the end of linear chromosomes in human cells and consist of repetitive (TTAGGG)n sequences in arrays of up to 25 kb [7]. Once telomeres become critically short, activation of cell arrest leads to replicative senescence, followed by apoptosis [8]. This mechanism represents a fundamental barrier to cancer initiation by limiting proliferation and maintaining genomic stability. The most important telomere lengthening mechanism requires telomerase, an enzyme that is overexpressed in approximately 90% of tumors, but is inactive in normal cells. Two key subunits necessary for maintaining the functionality of telomerase are Telomerase reverse transcriptase (TERT) and Cleft lip and palate transmembrane protein 1 (CLPTM1) [9–12]. A recent review provided the clinical value of polymorphisms in this gene and telomere length in lung cancer [13]. However, the results were inconsistent, and clinical significance of this polymorphism in Korean patients with NSCLC has not been studied. In this study, we investigated the clinicopathological and prognostic value of the polymorphism rs401681 in the TERT-CLPTM1 locus in NSCLC. And its association with telomere length was also investigated.

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2. Patients and Methods

2.1. Patients and tissue samples

Tumor specimens and corresponding normal lung tissue (n = 134) from January 2008 to June 2012 were provided by the National Biobank of Korea, Kyungpook National University Hospital (KNUH), supported by the Ministry of Health, Welfare and Family Affairs. All materials from the National Biobank of Korea, KNUH, were obtained under Institutional Review Board-approved protocols. Patients were enrolled in this study as the following criteria: they had a pathologic diagnosis of lung adenocarcinoma or squamous cell carcinoma, containing a minimum of 50% tumor cells and they had sufficient tissue for molecular analysis. Patients who underwent chemotherapy or radiotherapy prior to surgery were excluded. We collected basic clinical data including age, gender, disease stage, and smoking status. The pathologic staging of lung cancer was established according to the 7th AJCC staging system.

2.2. Single nucleotide polymorphism (SNP) genotyping

Peripheral blood samples were collected, and DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, USA). Polymerase chain reaction (PCR) amplification and analysis of the TERT-CLPTM1 locus for SNP determination were performed as described previously with minor modifications. PCR was performed using AmpliTaq Gold (Applied Biosystems, USA). The PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide to confirm the size of the bands. Direct DNA sequencing was performed using the ABI 3730 DNA sequencer by Bionics Inc, Korea.

2.3. Telomere length analysis

Tumor specimens and corresponding normal lung tissue were formalin fixed and paraffin embedded. Areas of interest containing representative tumor lesions were selected by the pathologist after review of the corresponding hematoxylin and eosin slides. Representative lesions from each case were marked on the paraffin blocks and manually cored with a 3.0-mm diameter cylindrical device. Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN). Telomere length was examined using a real-time PCR assay. For the quantitative determination of content relative to total DNA, primers for the specific amplification of telomeres and the β-globin gene were selected according to previous studies [14]. Real-time PCR was then carried out on the LightCycler 480 II system (Roche Diagnostics, Germany) with a total reaction volume of 20 μL. Each measurement was repeated in triplicate, and five serially diluted control samples were included in each experiment. The relative telomere length was calculated by the $2^{-\Delta\Delta CT}$ method. Briefly, telomere length was normalized against the β-globin gene, and then the telomere length of the tumor sample was compared to the mean telomere length in normal tissue and expressed as an n-fold ratio.

2.4. Statistical analysis

Chi-square test, Fisher's exact test, Mann-Whitney U test, and simple correlation analysis were used to analyze the relationship between variables. Survival curves, estimated with the Kaplan-Meier method (univariate analysis), were compared using the log-rank test. Overall survival was defined as the time from diagnosis to either death from disease or death from other causes. Disease-free survival was defined as the time from diagnosis to disease recurrence or development of distant metastasis. All p-values < 0.05 were considered statistically significant.

Table 1
Demographic and histopathological characteristics according to rs401681 polymorphisms in patients with non-small cell lung cancer.

Variables	n	rs401681 polymorphisms			p
		CC, n (%)	C/T, n (%)	TT, n (%)	
Study population	134	70 (52.2)	41 (30.6)	23 (17.2)	–
Gender					0.783
Male	95	51 (53.7)	29 (30.5)	15 (15.8)	
Female	39	19 (48.7)	12 (30.8)	8 (20.5)	
Age					0.875
< 60	66	33 (50.0)	21 (31.8)	12 (18.2)	
≥60	68	37 (54.4)	20 (29.4)	11 (16.2)	
TNM stage					0.476
Stage I	39	24 (61.5)	10 (25.6)	5 (12.8)	
Stage II	60	26 (43.3)	23 (38.3)	11 (18.3)	
Stage III	19	11 (57.90	4 (21.1)	4 (21.1)	
Stage IV	11	6 (54.5)	2 (18.2)	3 (27.3)	
Pathologic T stage					0.137
pT1	27	16 (59.3)	6 (22.2)	5 (18.5)	
pT2	90	41 (45.6)	32 (35.6)	17 (18.9)	
pT3	10	9 (90.0)	0 (0)	1 (10.0)	
pT4	5	3 (60.0)	2 (40.0)	0 (0)	
Pathologic N stage					0.296
N0	109	62 (56.9)	30 (27.5)	17 (15.6)	
N1	16	5 (31.3)	8 (50.0)	3 (18.8)	
N2	7	3 (42.9)	2 (28.6)	2 (28.6)	
Histology					0.206
Squamous cell carcinoma	64	32 (50.0)	24 (37.5)	8 (12.5)	
Adenocarcinoma	69	38 (55.1)	17 (24.6)	14 (20.3)	
Differentiation					0.965
Well or moderate	105	55 (52.4)	31 (29.5)	19 (18.1)	
Poor or undifferentiated	19	10 (52.6)	6 (31.6)	3 (15.8)	
Smoking					0.709
Yes/Ever	94	51 (54.3)	29 (30.9)	14 (14.9)	
Never	39	19 (48.7)	12 (30.8)	8 (20.5)	
EGFR mutation[a]					0.071
Negative	81	44 (54.3)	26 (32.1)	11 (13.6)	
Positive	29	15 (51.7)	5 (17.2)	9 (31.0)	

[a] CC + CT vs. TT, P = 0.037.

3. Results

3.1. Demographics of patients according to rs401681 genotype

The study population was composed of 95 men and 39 women with a median age of 61 years (range, 42–80 years). The genotype frequencies of the rs401681 polymorphisms were as follows: C/C, 52.2%; C/T, 30.6%; and T/T, 17.2%. The frequency of the rs401681C allele and T allele was 0.675 and 0.225, respectively. Clinicopathological characteristics of the rs401681 polymorphism in NSCLC are presented in Table 1. The rs401681 T allele tended to be associated with EGFR mutation (p = 0.071), though this was not statistically significant. When comparing between the C/C + C/T alleles and the T allele, EGFR mutation showed a strong correlation with the T allele (45.0% vs. 22.2%, p = 0.037). EGFR mutation was found in 36.4% (29/110), and 19del (n = 16) and L858R (n = 13) were reported. This polymorphism did not have any relationships with other clinical parameters.

Telomere length was analyzed in 134 patients with NSCLC. The average telomere length in NSCLC was 3.26-fold longer than that in matched normal tissues (SD = 0.48). Compared to normal tissues, the mean telomere lengths of NSCLC patients with CC, TT, and C/T alleles were 3.96-fold (SD = 0.73), 2.27-fold (SD = 0.68), and 2.44-fold (SD = 0.33) longer, respectively, and the differences were not significant (p = 0.113, Fig. 1). To further explore the correlation between telomere length and clinicopathological parameters in NSCLC, patients were categorized into two subgroups according to average telomere length (tumor to normal ratio, 3.26). The clinicopathological characteristics according to telomere length in NSCLC are summarized in Table 2. Telomere shortening was significantly found in Stage I and III