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F508 amino acid deletion mutation of CFTR gene in Korean lung cancer patients

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Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; RT-PCR, reverse transcription-polymerase chain reaction; DEPC, diethylpyrocarbonate; CF, cystic fibrosis

Abstract

Mutations of the transmembrane conductance regulator (CFTR) gene in cystic fibrosis lead to dysfunction of the lung, pancreas, and sweat glands, etc. To investigate the possibility of the relationship between lung cancer and the mutations of CFTR gene, we determined amino acid sequences using reverse transcription-polymerase chain reaction (RT-PCR) and DNA sequencing. In this study, the deletion mutation of 508th amino acid in one of nine lung caner patients was found confirming that CFTR gene mutation exists in a Korean lung cancer patient.

Keywords: CFTR, mutation, lung cancer

Introduction

Cystic fibrosis is a severe autosomal recessive genetic disorder affecting approximately 1 in 2,500 live births in the Caucasian population (Boat *et al.*, 1989). This disease invades a number of organs, including the lung, pancreas, and sweat glands (Boat *et al.*, 1989), and is characterized by recurrent pulmonary infections, impaired pulmonary function, and disseminated bronchiectasis. Transmembrane conductance regulator (CFTR) gene identified at chromosome 7 (Kerem *et al.*, 1989; Riordan *et al.*, 1989; Rommens *et al.,* 1989) is known to be responsible for cystic fibrosis, and a variety of mutations of CFTR gene have been identified (Xu and Gruenert, 1996; Sharer *et al.*, 1998; Cohn *et al.*, 1998).

Most frequent mutation in CFTR gene is 508th amino acid deletion and found in many diseases, including chronic pancreatitis (Cohn *et al.*, 1998; Sharer *et al.*, 1998), disseminated bronchiectasis (Pignatti *et al.*, 1995), bronchopulmonary aspergillosis (Weiner *et al.*, 1996), chronic bronchitis, and pulmonary emphysema (Bombieri *et al.*, 1998), etc. However, cystic fibrosis is not a common disease in Asian and only one case has been reported in a Korean male infant with recurrent bronchitis. (Moon HR *et al.*, 1988) It appears that there exists genetic characteristics among races.

In addition, CFTR gene mutation is found in pulmonary and breast cancer patients (Southey *et al.*, 1998), and it is hypothesized that CFTR gene may be related to cancer pathogenesis (Tien *et al.*, 1994). In this study, the analysis of CFTR gene in Korean lung cancer patients was made.

Materials and Methods

Specimens

In this experiment, samples were collected from patients who had been admitted to Wonju Christian Hospital in Korea. Nine patients with lung cancer on Histological examination particulated in this study.

RT-PCR (reverse transcription-polymerase chain reaction)

RNAs were extracted from cancer tissues by using RNaid kit (BIO 101, Vista, CA, USA). After ethanol precipitation, each RNA pellet was dissolved in 10 μL of DEPC (diethylpyrocarbonate)-treated distilled water and used for cDNA preparation.

cDNA synthesis was performed as described previously by Han *et al.* (2000). Two μL of purified RNA was reverse transcribed for 1 h at 42°C with 10 units of avian myeloblastosis virus reverse transcriptase, 2 μL of 10 × reaction buffer (100 mM Tris-HCl pH 8.8, 500 mM KCl, and 1% Triton X-100), 15 units of RNasin, 1 μg of oligo (dT) primer, 1 μL of 10 mM dNTP mix, and 2 μL of 25 mM magnesium chloride.

The cDNAs obtained by reverese transcription were amplified by PCR. The primer sequences for PCR were 5'-ACTGGAGCCTTCAGAGGGTA-3' for forward primer, and 5'-TGGCATGCTTTGATGACGCT-3' for backward

primer, respectively. PCR was performed in 20 μL of reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM potassium chloride, 2.5 mM magnesium chloride, 0.01% gelatin, 10 pmole of each primer, 100 μM of each deoxyribonucleotide triphosphates, and 1 unit of *Taq* DNA polymerase. The reaction was carried out using the modified method described by Saiki *et al*. (1988) in a Mini Cycler™ (MJ Research Inc., Watertown, MA, USA). Before cycling reaction, reaction tube was incubated at 94°C for 5 min to denature completely, and 30 cycling reactions were performed to amplify target sequences. Each reaction cycle included denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s and primer extension at 72°C for 30 s.

PCR products were subjected to agarose gel electrophoresis in Tris-acetate-EDTA buffer and visualized in the presence of ethidium bromide under ultraviolet transillumination.

DNA Sequencing

Amplified cDNAs were isolated from agarose gel and purified with DNA PrepMateTM (Bioneer, Chungwon, Korea). They were then subcloned by inserting the cDNA into a pGEM T-vector (Amersham Pharmacia Biotech., Uppsala, Sweden). Plasmids containing cDNA of CFTR were selected by α-complementation.

After growing the colonies, plasmid DNA was purified by using the modified method of Sambrook *et al.* (1989). DNA sequencing was performed by using the modified method of Sanger's dideoxy method (1978). A T7 DNA Polymerase Sequencing kit (Amersham Pharmacia Biotech., Sweden) was used for this process.

Clinical Findings

The clinical characteristics of nine patients were recorded on the basis of their clinical records.

Results and Discussion

The characteristics of nine patients used in this study were shown in Table 1. Patient No. 9 with adenocarcinoma of the lung was readmitted because squamous cell carcinoma was developed after left pneumonectomy and radical lymph node dissection. Patients No. 2 and No. 6 had anemia and pneumonia, respectively, and another six patients had no pertinent findings.

In the present study, a ΔF508 mutation was found in a Korean lung cancer patient on the basis of cDNA sequencing from 455th to 525th amino acid of CFTR protein. As shown in Figure 1, a F508 deletion of CFTR gene was appeared in patient No. 6. This is the first report of a mutated CFTR gene in Korean population. This case is thought to be a heterozygote because we could confirm four deletion mutations and two normal sequences among six clones (data not shown).

Among more than 900 kinds of CFTR gene mutations, ΔF508 is the most prevalent type. In normal Caucasian population, prevalence of heterozygote ΔF508 mutation can be reached up to 1.8% (Southery *et al*., 1998). Therefore, it is difficult to assume that ΔF508 mutation is associated with the pathogenesis of lung cancer. However, the presence of ΔF508 mutation in Korean population was a surprising result, since there was only one

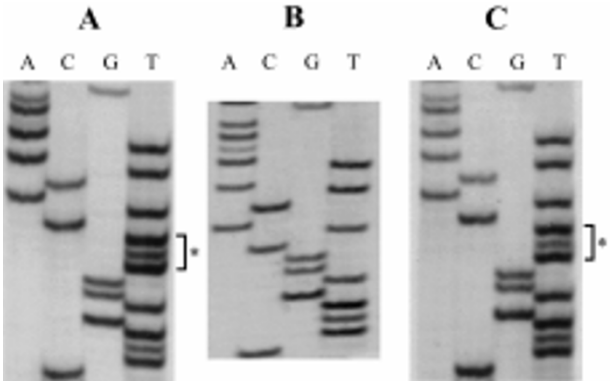

Figure 1. cDNA sequences around 508th amino acid of CFTR. * indicate the location of 508th amino acid (TTT). Panels A and C show normal sequences but panel B shows ΔF508 (TTT) mutant. Panels A, B, C correspond to patients No. 1, No. 6, No. 9 in Table 1, respectively.

Table 1. Clinical characteristics of nine lung cancer patients

No.	Age	Sex	Pathologic diagnosis	Stage	Accompanying disease	Smoking History
1	59	M	squamous cell carcinoma	IB	none	2 pack/day x 40 years
2	42	M	adenocarcinoma	IIB	anemia	none
3	68	M	squamous cell carcinoma	IIIA	none	1 pack/day x 40 years
4	43	F	adenocarcinoma with signet ring cell carcinoma	IIIA	none	none
5	71	M	adenocarcinoma	IIIA	none	1 pack/day x 40 years
6	27	F	bronchoalveolar carcinoma	IB	pneumonia	none
7	44	M	small cell carcinoma	extensive	none	2-3 pack/day x 20 years
8	61	M	squamous cell carcinoma	IB	none	1 pack/day x 40 years
9	60	M	adenocarcinoma	IIIB	squamous cell carcinoma	2-3 pack/day x 20 years

presumptive report of cystic fibrosis (CF) in Korea (Moon *et al*., 1988).

We analyzed 13 cDNA samples from 9 cancer patients and 4 normal lung tissues and ΔF508 CFTR gene was found only in one lung cancer patient. Although the sample size were is small, our results suggest two possibilities. First, there may exist a considerable rate of CFTR gene mutation in Korean population even though a typical CF presentation is rare. Recent studies revealed that the mutation in CFTR gene is not only associated with CF but also associated with many other epithelial disorders such as bronchiectasis, chronic bronchitis, and chronic pancreatitis. Considering the fact that epithelial disorders are also common in Korean population, a more large scale study searching for CFTR gene mutation is urgently needed. Second, the mutation in CFTR gene may be correlated with the lung cancer. Although this assumption is quite speculative, there are some supportive evidences. CFTR gene is needed for apoptosis in epithelial cells and mutated CFTR gene in epithelial cells loses their apoptotic control (Gottlieb, and Dosanjh. 1996). Indeed, Bombieri *et al.* (1998) found four different kinds of mutations in 26 lung cancer patients but there was no ΔF508 deletion mutant although it also needs a further verification by a large scale study.

In this study, the first case of CFTR gene mutation in a Korean lung cancer patient is reported. This No. 6 patient with left lower lobectomy for bronchoalveolar carcinoma was 27 years old and had chronic pneumonia for more than three years which was treated with antibiotics. A further, large scale study in Korean lung cancer patients as well as in normal populations is needed to identify the role of CFTR gene mutation.

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