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Analysis of EGFR gene mutations in tissue samples of lung cancer tumors

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Abstract

Lung cancer is the leading cause of cancer deaths worldwide. Approximately 25% of nonsmall-cell lung cancers have mutations in the *EGFR* gene, most of which occur in hotspot regions in exons 18, 19, 20, and 21. In-frame deletions in exon 19 (~50%) and the L858R point mutation in exon 21 (~40%) are associated with a favorable response to EGFR tyrosine kinase inhibitors. In this study, mutations of two exons of 19 and 21 in 50 lung cancer tumor samples were investigated by the sequence method. From 50 lung cancer patients, 8 (16%) patients had an L858R (c.2573T>G) mutation, 6 (12%) patients had deletion type 1a mutation, and one patient had deletion type 1b mutation. Examining the sequence of candidate genes associated with lung cancer can be very important in choosing the right treatment approach.

INTRODUCTION

Most lung cancer statistics include both small-cell lung cancer (SCLC) and nonsmall-cell lung cancer (NSCLC). In general, about 13% and 84% of all lung cancers are SCLC and NSCLC, respectively (1). Lung cancer is the second most common cancer, preceded only by prostate cancer in males and breast cancer in females. The clinical behavior of lung cancer is largely associated with its stage (2). The cure of the disease by surgery is only achieved in cases representing an early stage of lung cancer. Recent advances in the understanding of cell signaling pathways that control cell survival have identified genetic and regulatory aberrations that suppress cell death, promote cell division, and induce tumorogenesis (3). One such discovery is that of epidermal growth factor receptor (EGFR), a transmembrane receptor tyrosine kinase protein that is expressed in some normal epithelial, mesenchymal, and neurogenic tissue. The overexpression of EGFR has been reported and implicated in the pathogenesis of many human malignancies, including NSCLC (4). Some studies have shown that EGFR expression in NSCLC is associated with reduced survival (5). Approximately 25% of nonsmall-cell lung cancers have mutations in the *EGFR* gene, most of which occur in hotspot regions in exons 18, 19, 20, and 21. *EGFR* is activated by the binding of specific ligands, resulting in the activation of the RAS/mitogen-activated protein kinase (MAPK) pathway (6). EGFR-targeted therapies are FDA approved for use in treating patients with NSCLC who have previously failed to respond to traditional chemotherapy. Two oral anti-cancer drugs that inhibit EGFR, gefitinib (Iressa) and erlotinib (Tarceva), have recently been approved for use in treating advanced nonsmall-cell lung cancer, and mutations in EGFR have been discovered in association with some lung cancers. Since then, considerable effort has been made to identify clinical, morphologic, and molecular factors that can predict response rates to these drugs (7).

These mutations consist mainly of in-frame deletions in exon 19 (~50%) and the L858R point mutation in exon 21 (~40%) and are associated with a favorable response to EGFR tyrosine kinase inhibitors (EGFR-TKI), such as gefitinib and erlotinib (8). Exon 19 and 21 are located in the intracellular kinase domain of *EGFR*, which also includes exons 18 and 20. Insertions in exon 20 of *EGFR* have been reported to be associated with resistance to EGFR-TKI and poor prognosis in NSCLC patients (9). Single point mutations in exon 18 consist mainly of E709X and G719X mutations, which have been identified in several previous studies with limited sample sizes. However, other complex *EGFR* mutations including not only single point mutations in exon 18, but also other genetic alterations in the EGFR kinase domain have not been well characterized. In addition, the relationship between the complex mutations and sensitivity to EGFR-TKI therapy has not been completely elucidated (10).

METHOD AND MATERIALS

This study investigated 50 formalin-fixed paraffin-embedded (FFPE) tissue samples from 50 stage-two lung cancer patients, whose malignancy was confirmed by a pathologist. DNA was extracted from FFPE tissues using the GeneAll GenEx Tissue (GeneAll Korea) kit. For this purpose, 10 mg of tissue was first deparaffinized using xylene, and then the extraction process was continued according to the kit protocol. After examining the quality and quantity of the extracted DNA, exons 19 and 21 of the EGFR gene were amplified using nested PCR and Table 1 primers. The first cycle of amplifications were performed using a 5 min initial denaturation at 95 °C; followed by 30 cycles of 45 s at 95 °C, 45 s at 54 °C, 1 min at 72 °C, and a 6 min final extension at 72 °C. Production of the first cycle was amplified in the secondary cycle using the same conditions. The final products were cleared and sequenced with the internal primers using ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were analyzed using ChromasPro software and compared with the reference genome in the NCBI database.

Table 1. Primer sequences, exons 19 and 21

Primer name	Sequence
19 exon outer	5'-AAATAATCAGTGTGATTCGTGGAG-3' 5'-GAGGCCAGTGCTGTCTCTAAGG-3'
19 exon inner	5'-GTGCATCGCTGGTAACATCC-3' 5'-TGTGGAGATGAGCAGGGTCT-3'
21 exon outer	5'-GCAGCGGGTTACATCTTCTTTC-3' 5'-CAGCTCTGGCTCACACTACCAG-3'
21 exon inner	5'-GCTCAGAGCCTGGCATGAA-3' 5'-CATCCTCCCCTGCATGTGT-3'

RESULTS

The average age of the study population was 63 years. From the 50 included lung cancer patients, 28 were male and 22 were female. Eight (16%) patients (2 males and 6 females) had the L858R (c.2573T>G) mutation. Five of these patients were nonsmokers and three were smokers. All eight patients had adenocarcinoma; one was moderately differentiated, and seven were well differentiated. Thus, L858R mutation status was significantly correlated with gender and pathologic subtypes. From the 50 lung cancer patients, 6 (12%) patients (4 males and 2 females) had the deletion type 1a mutation (2,235-2,249 nucleotides deletion; deletion GGA ATT AAG AGA AGC). One patient (male) had the deletion type 1b mutation (2,236-2,250 nucleotides deletion; deletion GAATTAAGAGAAGCA). Thus, deletion 1a and 1b mutation statuses were not significantly correlated with any of the parameters studied.

Table 2. Clinical and pathological characteristics of patients and frequency of mutations

Variable	Parameter	N
Age	Mean~63	50
Gender	Female	22
	Male	28
Smoking history	Never	34
	Current	2
	Former	14
Metastasis	Yes	28
	No	22
Disease Stage	II	16
	III	11
	IV	23
Pathology subtype	Adenocarcinoma	14
	Non-adenocarcinoma	26
Mutations	L858R (exon 21)	8(16%)
	19-del 1a (exon19)	6(12%)
	19-del 1b (exon19)	1(2%)

DISCUSSION

Lung cancer is the leading cause of cancer deaths worldwide. The 2 major forms of lung cancer are nonsmall-cell and small cell lung cancers, which account for 85% and 15% of all lung cancers, respectively. Nonsmall-cell lung cancer can be divided into 3 major histologic subtypes: squamous cell carcinoma, adenocarcinoma, and large cell lung cancer (1, 11). Cigarette smoking causes all types of lung cancer, but it is most strongly linked with small cell lung cancer and squamous cell carcinoma (12). Adenocarcinoma is the most common type in patients who have never smoked. Nonsmall-cell lung cancer is often diagnosed at an advanced stage and has a poor prognosis (13). Cancer is the third main cause of death in Iran (27). According to GLOBOCAN 2012, although lung cancer is one of the major cancers in world, it is not considered as one of the most common cancers among Iranian men and women (14). Compared to other geographical locations, the incidence of lung cancer appears a little lower in Iran. A total of 623 genes with known or potential relationship to cancer have been sequenced in 188 human lung adenocarcinomas. Their analysis identified 26 genes that are mutated at significantly high frequencies and are probably involved in carcinogenesis. The frequently mutated genes include tyrosine kinases, among which are the EGFR homolog ERBB4, EPHA3, NTRK, APC, Rb1 and ets (15). Among these, the EGFR gene is of great importance in the choice of treatment approach (16). The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily, and it is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to the epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation (17). Mutations in this gene are associated with lung cancer. The EGFR gene has emerged as a critical therapeutic target, and the status of EGFR mutations has successfully guided clinical management (18). The presence of activating EGFR mutations, mainly in exons 18, 19, 20, and 21 which correspond to the tyrosine kinase domain, sensitizes lung adenocarcinomas to treatment with anti-EGFR tyrosine kinase inhibitors (TKi), such as erlotinib, gefitinib, afatinib, dacomitinib, and osimertinib (19). Basically, however, all TKi-treated lung adenocarcinoma patients will experience disease progression due to resistance mechanisms. The EGFR kinase inhibitors gefitinib and erlotinib are effective treatments for lung cancers with EGFR-activating mutations, but these tumors invariably develop drug resistance. Engelman et al. (2007) and Pao et al. (2004) found that in-frame deletions in exon 19 of the EGFR gene and somatic point mutations in codon 858 (exon 21) were common, particularly in lung cancers from “never smokers” and were associated, as found by others, with sensitivity to the tyrosine kinase inhibitors gefitinib and erlotinib. In this study, the exon 19 and 21 EGFR genes in 50 lung cancer tumor specimens were sequenced using Sanger sequencing (20-21).

From the 50 lung cancer patients, 8 (16%) patients had the L858R (c.2573T>G) mutation, 6 (12%) patients had deletion type 1a mutation (2,235-2,249 nucleotides deletion; deletion GGA ATT AAG AGA AGC), and one patient had deletion type 1b mutation (2,236-2,250 nucleotides deletion; deletion GAATTAAGAGAAGCA). Rosell et al. (2009) showed that mutations were deletions in exon 19 and L858R (19, 22). Median progression-free survival and overall survival for 217 patients who received erlotinib were 14 months and 27 months, respectively. Multivariate analysis showed an association between poor progression-free survival and the male sex and the presence of the L858R mutation as compared with a deletion in exon 19. The results suggested that EGFR-mutant lung cancer is a distinct class of nonsmall-cell lung cancer. Examining the sequence of candidate genes associated with lung cancer can be very important in choosing the right treatment approach.

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