MEKI gene mutation in Japanese lung adenocarcinoma patients

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Abstract. Recently, to identify potential somatic mutations in genes of the epidermal growth factor receptor (EGFR) signaling pathway, the MEKI gene mutation at exon 2 was identified. The mutant form of MEKI leads to the constitutive activity of extracellular signal-regulated kinase (ERK)-1/2. We investigated MEKI gene mutation status in 241 surgically treated lung adenocarcinoma cases from Nagoya City University Hospital. The presence or absence of the MEKI mutation was analyzed by direct sequencing. EGFR mutation status was previously investigated and reported. We detected only one case (0.4%) of the MEKI mutation (K57N) in our cohort. Total EGFR mutations were present in 101 patients (41.9%). The MEKI mutation was mutually exclusive with B-ras, K-ras and EGFR mutations. Thus, it is a rare mutation in Japanese lung cancer patients, and of limited value for lung adenocarcinoma.

Introduction

Lung cancer is a major contributor to malignant disease mortality due to its high incidence, malignant behavior, and the lack of major advancements in treatment strategy (1). The disease was the leading indication for respiratory surgery (42.2%) in 1998 in Japan (2), with more than 15,000 patients undergoing surgical procedures for lung cancer at Japanese institutions that year (2).

The mitogen-activated protein kinase (MAPK) pathway plays a critical role in the epidermal growth factor receptor (EGFR) signaling cascade. After the activation of EGFR signaling, key downstream steps involve the phosphorylation of the mitogen-activated protein kinase (MAPK) pathway, the MEK1/2, upstream of the ERK1/2 MAPK module (3). MEK1 is the main activator of both ERK1 and ERK2, as well as the critical isoform regulating tumor cell proliferation in vitro and in vivo (4). Approximately 25% of individuals with Cardiofaciocutaneous syndrome (CFC) have mutations in either the MEKI or MEK2 gene. These lead to increased MEK kinase activity, as judged by increased phosphorylation of its downstream effector ERK (5,6). Germline mutations of MEK are sensitive to MEK and RAF inhibition, suggesting the need for therapeutic options (7). More recently, the MEKI mutation at exon 2 was identified by mutational analysis of the EGFR signaling pathway genes in lung adenocarcinoma (8). MEKI mutations at exon 2 were also reported in cell lines, such as rat fibroblasts (9) and the ovarian cancer cell line ES-2 (10).

To determine MEKI status in Japanese lung adenocarcinoma patients for screening purposes, we investigated MEKI exon 2 mutation status by direct sequencing. The findings were compared to the clinicopathologic features of the lung adenocarcinoma patients.

Patients and methods

Patients. The study group included 241 lung adenocarcinoma patients who had undergone surgery at the Department of Surgery II, Nagoya City University Medical School, between 1997 and 2006. We started with 248 samples; however, 7 were defined as non-adenocarcinomas (4 squamous cell carcinomas and 3 adenosquamous carcinomas) upon retrospective pathological review. These 7 samples were wild-type (data not shown). Lung tumors were classified according to the general criteria for clinical and pathological recording of lung cancer in Japan. All tumor samples were immediately frozen and stored at -80˚C until assayed.

The clinical and pathological characteristics of the 241 lung adenocarcinoma patients were as follows: 151 cases at stage I, 31 at stage II and 59 at stage III-IV. The mean patient age was 64.8 years (range 38-83). Of the 241 lung adenocarcinoma patients, 139 (57.7%) were male and 102 (42.3%) were non-smokers. The samples from these patients had previously been sequenced for EGFR (11-14).

PCR assays for MEKI. Genomic DNA was extracted using the Wizard SV Genomic DNA Purification System (Promega) according to the manufacturer’s instructions. We then used 100 ng of each DNA for PCR analyses. The PCR reactions were performed using LA-Taq Kit (Takara Bio Inc., Shiga, Japan) in a 50-µl reaction volume. The primer sequences for the MEKI gene, exon 2 were as follows: forward 5-TTTCTT TCCATGATAGGAGT-3, reverse 5-ATCAGTCTTCCTTCT ACCCT-3. Cycling conditions were as follows: initial denaturation...
MEK1 gene mutation status in Japanese lung adenocarcinoma patients. Using the primer sets for MEK1 exon 2, we visualized the PCR products with 1% agarose gel. These samples were further studied. In our cohort, 1 of 241 patients harbored mutations in MEK1, as indicated by increased ERK phosphorylation as compared to controls (10). A transformation-competent mutant form of MEK1 was the main activator of both ERK1 and ERK2. In contrast, MEK2 removal had no impact on its own, but did cooperate with MEK1 ablation for the inhibition of ERK1/2 activity in LS174T colon carcinoma cells (4). Small molecule inhibitors of MEK would seem to be promising antitumor agents (18). In addition, a previous study exhibited the somatic mutation in MEK1 in human lung tumors, but not in colon, breast, prostate and pancreatic carcinomas, identified via mutational profiling of the genes encoding EGFR signaling pathway proteins in a large cohort of lung adenocarcinomas (8,19).

In a previous study, the same heterozygous mutation, K57N, was found in 2 of 207 lung adenocarcinomas. Neither of these two harbored mutations in other genes encoding components of the EGFR signaling pathway, such as EGFR, K-ras, B-raf or PIK3CA (8). Functional characterization of the mutant form in vitro indicated that its expression in 293T cells leads to constitutive activation of downstream signaling components (9). Thus, the MEK1 K57N mutant form displays gain-of-function properties (9). K57 is highly conserved among various species (8), and is located in a region between the nuclear export signal (amino acids 33-44) and the catalytic domain (amino acids 68-271) of MEK1. Consistent with the G-T mutation (a type of transversion known to be smoking-related), the sample was from a smoker. The K57N substitution led to constitutive activation of the MAPK pathway in vitro (Ba/F3 cells) (8).

A previous study identified germ-line MEK1 mutations in patients with CFC syndrome, a complex developmental disorder involving the heart, face and skin (5). Approximately 25% of individuals with CFC have mutations in either MEK1 or MEK2, which lead to increased MEK kinase activity, as judged by increased phosphorylation of its downstream effector MEK (6). These mutations include F53S, G128V and Y130C MEK1. The latter two of these occur in the kinase domain (5,20). The F53S mutant, like the Y130C mutant, is more active than wild-type protein in stimulating ERK phosphorylation (5). It is not yet clear if CFC patients are at an increased risk of cancer, but three affected individuals have developed neoplasms (21-23).

In summary, the MEK1 mutation may play a role in EGFR signaling. However, it is rare in Japanese lung cancer patients, and of limited value for lung adenocarcinoma.
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