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ORIGINAL ARTICLE

Mutational analysis of the kinase domain of *MYLK2* gene in common human cancers

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Abstract

Genetic alterations of the genes encoding protein kinases have been implicated in the development of human cancers. *Myosin light chain kinase 2, skeletal muscle* (*MYLK2*) encodes a calcium/calmodulin-dependent serine/threonine kinase. In a recent study, *MYLK2* gene was somatically mutated in colorectal carcinomas. The aim of this study was to explore the possibility that other common human carcinomas besides colorectal carcinomas harbored *MYLK2* mutations in the kinase domain. We analyzed exons 6 and 7 eccoding the kinase domain of *MYLK2* for somatic mutations in 60 gastric, 104 colorectal, 79 non-small cell lung, and 54 breast cancers using a polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP). We found one *MYLK2* mutation in lung adenocarcinomas, but not in other cancers. The *MYLK2* mutation detected was a missense mutation that would substitute an amino acid (E374D) However, there was no somatic mutation of the *MYLK2* gene. These data suggest that the kinase domain of *MYLK2* is rarely mutated in common human carcinomas and that it does not play a dominant role in cancer pathogenesis.

Keywords: *MYLK2*; Mutation; Cancer; Kinase

Introduction

Protein kinases regulate an intracellular signal-transduction pathway mediating cell proliferation, differentiation, and survival [7]. Human protein kinases consist of approximately 520 proteins and are subdivided into tyrosine- or serine/threonine-specific kinases [7]. Importantly, the protein kinase family is one of the most frequently mutated gene families found in human cancers, thus being a potential therapeutic target for human cancers [7].

Myosin light chain kinase 2, skeletal muscle (*MYLK2*) is a serine/threonine kinase that is exclusively expressed in skeletal and cardiac muscles, but the function of this protein in the cell is largely unknown [5]. Defects in *MYLK2* by the mutations are a cause of familial hypertrophic cardiomyopathy in humans [5]. The expression status and function of this protein in tumors remain unclear as well. Recently, Parsons et al. [10] analyzed 340 serine/threonine kinase genes in 204 colorectal cancer tissues for somatic mutations. They identified 23 mutations in eight genes, five of which (*MYLK2, MAP2K4, MYLK2, PAK4,* and *AKT2*) were mutated in more than one tumor. *MYLK2* gene mutations were found in 6 (2.9%) of 204 colorectal

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cancers. Of these, two *MYLK2* mutations were identified in the kinase domain. The kinase domains in the protein kinases are important for the functions, and mutations of these domains have been detected in human cancers [1,6,9]. It is interesting to see whether *MYLK2* kinase domain mutations occur in other types of cancer. However, data on the mutation status of *MYLK2* gene in human cancers besides colorectal cancers have been lacking to date. In this study, we analyzed 383 cases of common human carcinoma tissues of various origins for mutations in the kinase domain of *MYLK2* gene.

Materials and methods

Methacarn-fixed tissues of 60 gastric adenocarcinomas, 104 colorectal adenocarcinomas, 79 lung adenocarcinomas, and 54 breast invasive ductal carcinomas were randomly selected for this study. For this study, approval was obtained from the Catholic University of Korea, the institutional review board of the College of Medicine. Informed consent was provided according to the Declaration of Helsinki. The gastric carcinoma samples consisted of 24 diffuse-type, 20 intestinal-type, and 16 mixed-type gastric carcinomas according to Lauren's classification, and 12 early and 48 advanced gastric carcinomas according to the depth of invasion. The male to female ratio was 36:24. Patient age ranged from 35 to 80 years, with an average of 55.6 years. The TNM stages of the gastric cancers were stage 0 ($n = 8$), stage I ($n = 17$), stage II ($n = 15$), stage III ($n = 14$), and stage IV ($n = 6$). The colorectal carcinomas originated from cecum ($n = 2$), ascending colon ($n = 19$), transverse colon ($n = 6$), descending colon ($n = 4$), sigmoid colon ($n = 28$), and rectum ($n = 45$). The male to female ratio was 57:47. Patient age ranged from 37 to 80 years, with an average of 57.3 years. The TNM stages of the colorectal carcinomas were stage I ($n = 10$), stage II ($n = 48$), stage III ($n = 36$), and stage IV ($n = 10$). The breast carcinomas consisted of 8 intraductal and 46 invasive ductal carcinomas. The male to female ratio was 54:0. Patient age ranged from 27 to 73 years, with an average of 45.6 years. The TNM stages of the breast carcinomas were stage 0 ($n = 8$), stage I ($n = 8$), stage II ($n = 18$), and stage III ($n = 20$). The lung adenocarcinoma tissue samples consisted of 46 pure adenocarcinomas, 30 adenocarcinomas with features of bronchioloalveolar carcinoma, and 3 pure bronchioloalveolar carcinomas. The male to female ratio of the patients was 43:36. Patient age ranged from 37 to 78 years, with an average of 59.0 years. The patients consisted of 27 current smokers, seven former smokers, and 45 non-smokers. The TNM stages of the lung adenocarcinomas were stage IA ($n = 26$), stage IB ($n = 25$), stage IIA ($n = 14$), stage IIB ($n = 12$), and stage IIIA ($n = 2$). In this study, primary lesions, but not the metastatic lesions, were analyzed for mutations.

Malignant cells and normal cells were selectively procured from hematoxylin- and eosin-stained slides by microdissection using a 301/2 gauge hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) affixed to a micromanipulator as described previously [8]. DNA was extracted by a modified single-step DNA extraction method as described previously [8]. Because the *MYLK2* kinase domain mutations in the colorectal cancers were detected only within exons 6 and 7 in the previous study [10], genomic DNA each from tumor cells and corresponding normal cells of the same patients were amplified with 2 primer pairs covering the DNA sequences in exons 6 and 7 using polymerase chain reaction (PCR). The primer sequences were as follows (forward and reverse, respectively): exon 6 (5′ -taccttgacttccctggtc -3′ and 5′-ccactacttcgggcactcac -3′) and exon 7 (5′- accaccaggcacg-gagcaag -3′ and 5′- cccaggaggccccagtctg -3′). Numbering of cDNA of *MYLK2* was done with respect to the ATG start codon (NM_033118). Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection using single-strand conformation polymorphism (SSCP) autoradiogram. PCR and SSCP analyses were performed as described previously [8].

Results and discussion

Genomic DNAs isolated from normal and tumor tissues through microdissection were analyzed for mutations in exons 6 and 7 encoding the kinase domain of the *MYLK2* gene by PCR-SSCP analysis. All of the PCR products were clearly seen on the SSCP autoradiograms. However, SSCP of the whole samples analyzed did not reveal any aberrantly migrating band compared to the wild-type bands of the normal tissues (Fig. 1). To confirm the SSCP results, we repeated the experiments twice, including tissue microdissection, PCR, SSCP, and direct DNA sequencing analysis, to ensure the specificity of the results, and found that the data were consistent (data not shown).

Because genetic alterations of protein kinase-encoding genes profoundly contribute to the development of cancers and could be the therapeutic targets in the treatment of cancer patients [7], we tried to find *MYLK2* mutations in common human carcinomas in the present study. Moreover, because the previous study showed a modest frequency (2.9%) of *MYLK2* mutation in the colorectal carcinomas [10], we expected to detect some mutations at least in the colorectal carcinoma samples. However, we detected no *MYLK2* kinase domain mutation in the samples, suggesting that *MYLK2* kinase domain mutation in exons 6 and 7 is rare in common