No Evidence of Peutz-Jeghers Syndrome Gene LKB1 Involvement in Left-sided Colorectal Carcinomas


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Abstract

LKB1 serine/threonine kinase is a gene for Peutz-Jeghers cancer predisposition syndrome. Most studies have detected a low frequency of LKB1 defects in sporadic cancer. A notable exception is a recent report describing frequent, mostly missense type, LKB1 mutations in Korean distal colorectal tumors. To clarify the role of LKB1 in colon cancer, we scrutinized 50 left-sided Korean and Finnish specimens. No somatic mutations were found. The seven Korean somatic missense mutations reported previously were functionally analyzed, and five were found not to alter LKB1 kinase activity. One of these changes was found to be a germ-line polymorphism. LKB1 involvement in distal colorectal cancer is not common.

Introduction

Germ-line mutations of the serine/threonine kinase gene LKB1 cause PJS\(^1\) (1, 2), which is characterized by hamartomatous polyposis of the gastrointestinal tract and mucocutaneous pigmentation (3–5). Patients with PJS are also known to have a significantly increased risk of cancer as compared with the normal population (6, 7). A wide spectrum of neoplasia, particularly cancers of the gastrointestinal tract (stomach, small intestine, and colon), pancreas, breast, testis, and ovary appears to be associated with PJS (5–7).

Most of the detected germ-line LKB1 mutations lead to truncated protein products (1, 2, 8, 9). Loss-of-function type mutations and the observation of loss of heterozygosity in polyps of PJS patients indicate that LKB1 acts as a tumor suppressor gene (10), and further studies have confirmed this notion (9, 11, 12). These findings indicate that LKB1 might possibly be involved in the pathogenesis of sporadic cancers as well. However, somatic mutations appear to be rare, at least in breast, colorectal, gastric, testicular, pancreatic, and ovarian cancer, as well as in malignant melanoma (13–20). A notable exception has been a recent report by Dong et al. (21). These authors had detected a high frequency of somatic mutations in Korean left-sided colorectal tumors (10 of 19, 53.8%) and left-sided adenomas with high-grade dysplasia (2 of 7, 28.6%), highlighting LKB1 as an important gene contributing to sporadic colorectal tumorigenesis.

Because we and others (14, 16, 17) had found little evidence for LKB1 involvement in sporadic colorectal cancer, we investigated a series of 21 Finnish and 29 Korean distal colorectal cancer samples for LKB1 mutations to clarify the role of this gene in colonic tumor initiation and progression. Furthermore, we analyzed the functional consequences of the seven missense mutations that were reported previously in left-sided colon cancer (21) to evaluate their possible effect on LKB1 kinase function.

Materials and Methods

A series of 21 Finnish and 29 Korean left-sided sporadic colorectal carcinomas were obtained for LKB1 screening. Previous work had proposed that LKB1 mutations are not an early change in colorectal tumorigenesis (21). Thus, in this work, it was ensured that a considerable proportion of the lesions were well advanced, although no other selection criteria were introduced. The stage distribution of the samples according to Duke’s classification was: A, 3 (6%); B, 10 (20%); C, 20 (40%); and D, 17 (34%). Mutation analyses were typically performed using direct genomic sequencing; the original goal was to analyze approximately 20 Finnish and 20 Korean cases using this resource-demanding method (final numbers, 21 and 23, respectively). SSCP was used to evaluate the remaining six Korean samples. The conditions for SSCP and sequencing are described by Avizienyte et al. (14, 15).

To further evaluate an LKB1 missense variant (F354L) present in both of the studied populations, ASO was performed. Genomic DNA samples were amplified either with primers used in SSCP or direct sequencing. PCR products were run in 2% agarose (NuSieve) gel to verify the amplification, and thus avoiding the need for hybridization with a wild-type probe. PCR products from three individuals were pooled together. Slot-blot were made on nylon membranes using a vacuum manifold. Probe was labeled with [\(^{32}\)P]-dATP using T4-poly nucleotide kinase (New England BioLabs). Filters were hybridized at 62°C with a probe containing the mutant sequence (5'-GGA CCT TTT GGA CAT CGA G-3'). If a positive signal was obtained, the respective samples were rehybridized separately on a new membrane. Positive results were verified by genomic sequencing.

Altogether 299 normal tissue DNA specimens from Finnish colorectal carcinoma patients and 84 anonymous cancer-free blood donors were analyzed. To evaluate the frequency of the alteration in the Korean population, normal tissue DNA derived from 50 colorectal cancer patients and 36 cancer-free blood donors were obtained for ASO screening.

The protein kinase activity studies of four missense type mutations were based on an autophosphorylation assay that has been described previously (22). The expression plasmid DNA, pCDNA3/LKB1 myc, which contained the wild-type LKB1 coding sequence and c-Myc epitope tag (EQKLISEEDL) on its COOH terminus, was constructed as described previously (11). Using this plasmid DNA as template, each of the mutant LKB1 expression plasmids was generated by in vitro mutagenesis using the GeneEditor in vitro site-directed mutagenesis system (Promega), according to the procedure suggested by the manufacturer. The data on mutagenic oligonucleotides used is available on request.

Results and Discussion

A total of 21 Finnish left-sided colorectal carcinomas were first screened for LKB1. In this series, only one missense change was...
detected in the coding sequences and exon/intron boundaries. All of the other changes were previously reported intronic polymorphisms. The observed missense change converts phenylalanine to leucine at codon 354 (F354L). This variant was also seen in normal tissue of the patient, indicating a germ-line alteration (Fig. 1). Thus, no somatic alterations were detected. Interestingly, the germ-line missense change was identical to one of the somatic mutations reported by Dong et al. (21).

To further evaluate the missense variant, we performed normal tissue DNA ASO screening of 299 unscreened Finnish colorectal carcinoma patients. None of these displayed the change. Taken together, only 1 of 320 (0.3%) Finnish colorectal carcinoma patients displayed F354L. For this variant, we also analyzed 84 Finnish cancer-free controls, and none of these carried the change.

Somatic mutations in \( LKB1 \) coding region were not detected in the studied 29 Korean distal colorectal carcinoma cases. In addition to intronic polymorphisms, four samples displaying heterozygous F354L missense type change (14%), presumably a germ-line variant as in one of the Finnish patients, were detected. To confirm the presence of this germ-line variant in the Korean population, we screened normal tissue DNA from 50 additional colorectal cancer patients and 36 cancer-free individuals for germ-line \( LKB1 \) F354L by ASO. Heterozygous F354L (confirmed by sequencing) was observed in one carcinoma patient and in two controls (Fig. 2). The overall frequency of F354L variant in Korean colorectal carcinoma patients was 6.3% (5 of 79) and 5.6% (2 of 36) in Korean cancer-free controls. This result suggests that the variant is a neutral polymorphism.

We next studied protein kinase activity of the seven missense changes (G171S, E199K, D208N, G215D, P281L, F354L, and T367M) reported as somatic mutations in a previous study focusing on Korean left-sided colorectal carcinomas (Ref. 21; Fig. 3). Unexpectedly, in the autophosphorylation assay, normal activity was observed in five of seven of the missense type mutations, suggesting that these changes do not alter the kinase activity of \( LKB1 \). Five of the changes,

![Fig. 1. Heterozygous \( LKB1 \) missense germ-line variant detected in Finnish and Korean populations [F354L (C→G); arrow].](image)

![Fig. 2. F354 (C→G) detected using ASO. A positive control (1) was included for all hybridizations. In the first round, three samples were pooled together, and samples from positive pools (4) were subsequently hybridized individually. Finally, samples positive by ASO were sequenced to confirm the presence of heterozygous F354 (C→G).](image)

LKB1

![Fig. 3. The protein kinase activity assay of the seven previously reported missense changes. Five variants (G171S, E199K, D208N, F354L, and T367M) displayed normal kinase function. K78F served as a negative control (kinase-dead mutant).](image)

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