Mutational Analysis of **STK11** Gene in Ovarian Carcinomas

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Recently **STK11**, the causative gene of Peutz-Jeghers syndrome (PJS) was identified on chromosome 19p13.3. PJS is often accompanied by several malignancies, including breast tumor, adenoma malignum of the uterine cervix, and ovarian tumor. To investigate the involvement of **STK11** gene in the development of ovarian carcinomas, we analyzed 30 ovarian carcinomas for loss of heterozygosity (LOH) in 19p near the **STK11** gene of PJS. LOH analysis was performed on 19p near the **STK11** gene of PJS. We also detected LOH in 2 (11%) of 19 informative ovarian carcinomas. Our results suggest that mutations of the **STK11** gene may play a limited role in the development of ovarian carcinomas.

Key words: **STK11** — Peutz-Jeghers syndrome — Human ovarian carcinoma — LOH study — Mutation search

Peutz-Jeghers syndrome (PJS) is an autosomal dominant hereditary disease characterized by hamartomatous polyposis in the stomach, small intestine, and colon, as well as mucocutaneous pigmentation.1,2) Previous studies reported that PJS is often accompanied with a higher incidence of other malignancies, such as breast cancer,3) adenoma malignum of the uterine cervix,4) and ovarian tumors,5) and that patients with PJS have an 18-fold higher risk of malignancy than the general population.6) Recently, the PJS gene was identified7,8) and named **STK11**; it encodes a novel serine/threonine kinase and is located on chromosome 19p13.3. Truncating germline mutations were identified in patients with PJS in both Caucasians and Japanese.8,9) The **STK11** gene is thought to act as a tumor suppressor gene, as evidenced by loss of heterozygosity (LOH) in PJS polyps.10) In sporadic tumors, **STK11** gene mutations were observed in colon cancer12) and testicular tumor,13) though no mutation has been reported in breast cancer.14) Sato et al.15) reported allelic deletions of chromosome 19p in one-third of ovarian carcinomas, so we performed LOH analysis on 19p near the **STK11** locus and mutational analysis of the **STK11** gene of 30 ovarian carcinomas of sporadic form in order to determine whether **STK11** genetic alterations contribute to the development of ovarian carcinomas.

MATERIALS AND METHODS

Preparation of DNAs Materials used in this study were obtained during the course of surgical treatments at Sapporo Medical University Hospital. We examined 30 fresh frozen samples of various types (22 serous type, 4 clear cell type, 1 endometrioid type). DNAs were extracted from fresh frozen samples according to methods described elsewhere.16) Histological diagnosis of each tumor was classified according to the WHO classification.17) and clinical stage was determined according to the International Federation of Gynecology and Obstetrics.18) LOH analysis Matched pairs of normal and tumor genomic DNA of each sample were analyzed for LOH with a microsatellite marker mapped on 19p13.3, D19S886. Polymerase chain reaction (PCR) products were subjected to electrophoresis, followed by staining of the gel with SYBR green I (FMC BioProducts, Rockland, ME) as described elsewhere.19) PCR single-strand conformation polymorphism (PCR-SSCP) and nucleotide sequence analysis All samples were screened for genetic alterations of exons 1–9 containing the entire coding region of **STK11** by PCR-based SSCP analysis. PCR primers used in this study were described previously.20) PCR amplification was carried out in 20 µl of each reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.2 µM of each dNTP, 0.4 mM of each primer, 50 ng of genomic DNA and 0.5 units of TaKaRa Taq (a recombinant Taq DNA polymerase, TaKaRa, Otsu). Mixtures were heated to 94°C for 2 min, and then cycled 35 times; each cycle consisted of denaturation at 94°C for 30 s, annealing at 58–66°C for 30 s, and strand elongation at 72°C for 30 s. PCR products were denatured and subjected to electrophoresis in 12% polyacrylamide gel (ratio of acrylamide/bis-acrylamide, 39:1) with 10% glycerol at 17°C. After...
electrophoresis, gels were stained with SYBR green II (FMC BioProducts). The nucleotide sequence of aberrant PCR products was directly determined using an Applied Biosystems model 377 DNA sequencer (Perkin-Elmer Cetus, Norwalk, CT) with a PCR primer and a Dye terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA). Each mutation was verified in both the sense and antisense directions.

RESULTS

We conducted LOH analysis of pairs of 30 ovarian carcinomas, by using a microsatellite marker, D19S886, located on 19p13.3. Two (11%) of 19 informative ovarian carcinomas showed LOH at this loci (Fig. 1A). No second-hit mutations were found in the coding region of the \textit{STK11} gene in these two cases with deletion.

To screen alterations of the \textit{STK11} gene in 30 primary ovarian carcinomas, we performed PCR-SSCP analysis of the entire coding region of \textit{STK11} gene. In one case (T-32, 3.3%), an aberrant SSCP pattern in exon 6 was detected. Nucleotide sequence analysis of this case revealed a missense mutation at codon 281 (CCG to CTG) that would result in a substitution of Pro to Leu in the predicted protein (Fig.1B). The somatic nature of this mutation was established by nucleotide sequencing derived from normal tissue of the same patient (data not shown). A microsatellite marker at the D19S886 locus was retained in T-32, and direct nucleotide sequencing revealed no significant loss of normal C in the T-32 tumor. Our previous data\textsuperscript{20} indicated complete LOH on the TP53 locus of T-32, excluding the possibility of contamination with normal tissue, suggesting that mutation affected one of the two alleles. These results suggest that the status of this mutation was somatic and heterozygous. To characterize the role of this mutation in ovarian carcinoma, the clinicopathological status of this case (T-32) was analyzed, as regards onset age, FIGO Stage, histology, and familial malignancies. The patient (T-32) was 45 years old, stage IV, clear cell carcinoma, and there were no familial malignancies within the second-degree relatives.

Fig. 1. A. Representative example of LOH on D19S886 locus in T-25. T and N: paired DNA samples isolated from tumor and normal tissue. The tumor sample shows loss of one allele when compared to the normal heterozygote. B. \textit{STK11} mutation in ovarian carcinoma. An arrow indicates nucleotide change (CCG to CTG) at codon 281 in T-32.
DISCUSSION

This paper reports a search for STK11 gene mutations in ovarian carcinomas, although the sample size was limited. We found a low frequency of LOH at D19S886 and infrequent somatic mutation of STK11 in ovarian carcinomas. Similar results were observed in sporadic colon, breast, and testicular tumors. On the other hand, Dong et al. reported that left-side colon cancer had frequent genetic alterations in both alleles of the STK11 gene; they suggested that STK11 is a tumor suppressor gene and that genetic alterations of STK11 could contribute to development of left-side colon cancer. However, we were not able to detect “two-hit” mutation in our case (T-32) with STK11 mutation; nucleotide sequencing of T-32 revealed both mutant (CTG) and normal (CCG) alleles. Somatic and heterozygous mutations of STK11 were found in testicular and colon tumor, including the same mutation at codon 281 (CCG to CTG) that we identified in this study. Nakagawa et al. also reported that frequent mutations in PJS patients occurred at the mononucleotide-repeat region (CCCCCC) corresponding to codons 279–281, which includes the mutation in this study. Some of the mutations clustered in this region might be associated with a defect of DNAs mismatch repair systems, although our previous results showed that T-32 revealed no RER(+) phenotype. Therefore, we consider that somatic mutation in one allele of STK11 may contribute to the development of a subset of ovarian carcinomas.

These data do not exclude the possibility of the STK11 gene being inactivated through genetic alterations, since the mutation screening approach used does not allow the detection of some mutation types, such as large genomic deletion. Furthermore, PJS has been reported to be accompanied with sex stromal ovarian tumors, but we examined only epithelial ovarian carcinomas. The role of STK11 genetic alterations in other types of ovarian tumor should be examined. Our results suggest that somatic and heterozygous mutation of STK11 gene may play a limited role in the development of epithelial ovarian carcinoma.

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REFERENCES

1) Peutz, J. L. A. On a very remarkable case of familial polyposis of the mucous membrane of the intestinal tract and nasopharynx accompanied by peculiar pigmentation of the skin and mucous membrane. Ned. Tijdschr. Geneeskd., 10, 134–146 (1921).
2) Jeghers, H., McMchick, V. A. and Katz, K. N. Generalized intestinal polyposis and melanin spot of the oral mucosa, lips and digits. N. Engl. J. Med., 241, 1031–1036 (1946).
3) Trau, H., Schewach-Millet, M., Fisher, B. K. and Tsur, H. Peutz-Jeghers syndrome and bilateral breast carcinoma. Cancer, 50, 788–792 (1982).
4) Podczaski, E., Kaminski, P. F., Pees, R. C., Singapuri, K. and Sorosky, J. I. Peutz-Jeghers syndrome with ovarian sex cord tumor with annular tubules and cervical adenoma malignum. Gynecol. Oncol., 42, 74–78 (1991).
5) Cantu, J. M., Rivera, H. and Ocampo-Campos, R. Peutz Jeghers syndrome with feminizing Sertoli-cell tumor. Cancer, 46, 223–228 (1980).
6) Young, R. H., Welch, W. R., Dickerson, G. E. and Scully, R. E. Ovarian sex stromal tumor with annular tubules. Review of 74 cases including 27 with Peutz-Jeghers syndrome and 4 with adenoma malignum of the cervix. Cancer, 50, 1384–1402 (1982).
7) Giardiello, F. M., Welsh, S. B., Hamilton, S. R., Offerhaus, G. J. A., Gittelsohn, A. M., Booker, S. V., Krush, A. J., Yardeley, J. H. and Luk, G. D. Increased risk of cancer in the Peutz-Jeghers syndrome. N. Engl. J. Med., 316, 1511–1514 (1987).
8) Hemminki, A., Markie, D., Tomlison, I., Avizienyte, E., Roth, S., Loukola, A., Bignell, G., Warren, W., Aminoff, M., Houglund, P., Jarvinen, H., Kistoo, P., Pelin, K., Ridanpaa, M., Salovaara, R., Torio, T., Bodmer, W., Olshwang, S., Osln, S. A., Stratton, R. M., Chapelle, A. and Aaltonen, A. L. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature, 391, 184–187 (1998).
9) Jenne, E. D., Reimann, H., Nezu, J., Fridel, W., Loff, S., Jescske, R., Mullar, O., Back, W. and Zimmer, M. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat. Genet., 18, 38–43 (1998).
10) Nakagawa, H., Koyama, K., Miyoshi, Y., Ando, H., Baba, S., Watatani, M., Yasutomi, M., Matsuura, N., Monden, M. and Nakamura, Y. Nine novel germline mutations of STK11 in ten families with Peutz-Jeghers syndrome. Hum. Genet., 103, 168–172 (1998).
11) Hemminki, A., Tomlison, I., Markie, D., Jarvinen, H., Sistonen, P., Bjorkqvist, A.-M., Knufitila, S., Salovaara, R., Bodmer, W., Shibata, D., Chapelle, A. and Aaltonen, L. A. Localization of a susceptibility locus for Peutz-Jeghers disease to 19p using comparative genomic hybridization and targeted linkage analysis. Nat. Genet., 15, 87–90 (1997).
12) Dong, S. M., Kim, K. M., Kim, S. Y., Shin, M. S., Na, E. Y., Lee, S. H., Park, W. S., Yoo, N. J., Jang, J. I., Yoon, C. Y., Kim, J. W., Kim, S. Y., Yang, Y. M., Kim, S. H., Kim, S. H. and Lee, J. Y. Frequent somatic mutations in serine/threonine kinase 11/Peutz-Jeghers syndrome gene in left side colon cancer. Cancer Res., 58, 3787–3790
13) Avizienyte, E., Roth, S., Loukola, A., Hemminki, A., Lothe, R. A., Stenwig, A. E., Fossa, S. D., Salovaara, R. and Aaltonen, L. A. Somatic mutations in LKB1 are rare in sporadic colorectal and testicular tumors. *Cancer Res.*, **58**, 2087–2090 (1998).

14) Bignell, G. R., Barfoot, R., Seal, S., Collins, N., Warren, W. and Stratton, M. R. Low frequency of somatic mutations in the LKB1/Peutz-Jeghers syndrome gene in sporadic breast cancer. *Cancer Res.*, **58**, 1384–1386 (1998).

15) Sato, T., Saito, H., Morita, R., Koi, S., Lee, J. H. and Nakamura, Y. Allelotype of ovarian cancer. *Cancer Res.*, **51**, 5118–5122 (1991).

16) Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G. and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.*, **50**, 7184–7189 (1990).

17) Scully, R. E., Bonfiglo, T. A., Kurman, R. J., Silverberg, S. G. and Wilkinson, E. J. Histological typing of female genital tract tumors. In “International Histological Classification of Tumors,” 2nd Ed., WHO (1996). Springer-Verlag, Berlin.

18) FIGO Stages—1988 Revision. *Gynecol. Oncol.*

19) White, H. W. and Kusukawa, N. Agarose-based system for separation of short tandem repeat loci. *Biotechniques*, **22**, 976–980 (1997).

20) Kobayashi, K., Sagae, S., Kudo, R., Saito, H., Koi, S. and Nakamura, Y. Microsatellite instability in endometrial carcinomas. *Genes Chromosom. Cancer*, **14**, 128–132 (1995).