Advances in Brief

Mutation of K-ras Protooncogene Is Associated with Histological Subtypes in Human Mucinous Ovarian Tumors

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Abstract

A series of 57 mucinous and 47 serous ovarian tumors (adenomas, tumors of borderline malignancy, and carcinomas) were examined by polymerase chain reaction-single strand conformation polymorphism analysis and direct sequencing for mutations in codons 12, 13, and 61 of K-ras gene. Higher incidence of K-ras mutations was observed in mucinous tumors compared to serous ones. Mutations were detected in 4 of 30 mucinous adenomas (13%), in 4 of 12 mucinous tumors of borderline malignancy (33%), and in 7 of 15 mucinous carcinomas (46%). Only 1 of 17 serous carcinomas (6%) had a mutation of K-ras in serous ovarian tumors. All mutations identified were in codon 12. Detailed analysis revealed that more K-ras mutations in mucinous adenomas were observed in intestinal type (identified in 4 of 13) than in endocervical type (identified in 0 of 17). Thus, K-ras gene codon 12 mutations in mucinous ovarian adenomas appear to be associated with the occurrence of intestinal type adenomas.

Introduction

A series of genetic alterations detected in premalignant states of carcinomas and in malignant tumors provide important information about the mechanism of carcinogenesis (1). In colorectal neoplasia, alterations in the cellular protooncogenes leading to disordered cell growth and differentiation have been identified (1, 2). The common epithelial ovarian neoplasms are classified on histogenetic principles mainly in serous, mucinous, endometrioid, and clear cell tumors. The origin of serous ovarian tumors is the surface epithelium (3). Mucinous ovarian tumors are considered to originate from the ovarian surface epithelium (a müllerian origin) or from a teratoma, but this histogenesis of the mucinous ovarian tumors has not been clearly elucidated (3). Recently, mutations of K-ras gene have been detected more frequently in mucinous ovarian carcinomas and tumors of borderline malignancy than in serous ones (4). But the association of K-ras mutations with histological subtypes of the ovarian mucinous tumors has not been determined. Additionally, the genetic alterations involved in ovarian adenomas are still poorly understood. In the present study, we investigated the possible involvement of K-ras mutations in the development of mucinous ovarian adenomas, tumors of borderline malignancy and carcinomas, and serous tumors, and discuss the association of K-ras mutations with histological subtypes in mucinous ovarian tumors.

Materials and Methods

Specimens. A total of 52 cases of ovarian adenomas (30 mucinous and 22 serous tumors), 20 ovarian tumors of borderline malignancy (12 mucinous and 8 serous tumors), and 32 ovarian adenocarcinomas (15 mucinous and 17 serous tumors) were analyzed in this study. All the specimens were obtained from the University of Tsukuba Hospital (Ibaraki, Japan). Before DNA extraction from these specimens, histological diagnosis was confirmed by microscopic examination of the hematoxylin-eosin-stained sections according to the WHO criteria (5). All cases of mucinous adenomas and tumors of borderline malignancy were classified into two types according to the lining of the epithelium. The intestinal-type epithelium contains goblet cells and resembles the epithelium of the intestine or stomach. The endocervical-type epithelium resembles the epithelium of the endocervix of the uterus. The samples containing both types of epithelium in the same section were excluded.

DNA Extraction. The 10-μm thick sections from formalin-fixed paraffin-embedded tissue blocks were deparaffinized by washing with xylene followed by absolute ethanol. The deparaffinized tissues were incubated overnight at 55°C in 30 μl of digestion buffer (1 mg/ml proteinase K, 0.1 M Tris-HCl, pH 8.8). Then DNA was extracted by twice adding an equal volume of phenol/chloroform/isoamylalcohol mixture and was recovered by ethanol precipitation.

PCR-SSCP Analysis. Mutation of K-ras gene was examined by using PCR-SSCP analysis (6) with slight modifications. Two sequences containing K-ras mutations with histological subtypes

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The abbreviations used are: PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism.

Results

Ovarian mucinous and serous adenomas, tumors of borderline malignancy, and carcinomas were examined for point mutations in codon 12, 13, or 61 of K-ras gene by PCR-SSCP analysis. The mobility patterns in the SSCP gel are shown in Fig. 1. Mobility shifts indicating...
The presence of point mutations in codon 12 or 13 were found in 4 of 30 mucinous adenomas (13%), in 4 of 12 mucinous tumors of borderline malignancy (33%), and in 7 of 15 mucinous carcinomas (46%). In serous tumors, however, only 1 of 17 carcinomas (6%) showed an altered migration. All other serous adenomas, tumors of borderline malignancy, and carcinomas represented the wild-type pattern. In all cases examined, no altered migration indicating the presence of a point mutation in codon 61 was detected.

Every case showing a mobility shift in SSCP was examined by direct sequencing to confirm a mutation. There was a complete concordance between the mobility patterns in SSCP and the results of direct sequencing. Typical DNA sequencing autoradiographs are shown in Fig. 2. The results are summarized in Table 1. All 16 cases confirmed by sequencing involved a second base change of codon 12. Eight cases involved a base change from GGT to GTT (Gly to Val), 7 from GGT to GAT (Gly to Asp), and 1 from GGT to GCT (Gly to Ala). A high frequency of K-ras mutation in mucinous tumors including adenomas compared to serous tumors was observed. K-ras mutations were detected in stages Ia and Ib of mucinous tumors of borderline malignancy and carcinomas (Table 1). Two cases, cases 43 and 44, which showed stage IIIc and IIa, respectively, involved a base change from GGT to GTT. Only one case of serous carcinoma with a K-ras mutation was in stage Ia (case 88).

Analysis of correlation between histological subtypes of ovarian tumors and K-ras mutations is summarized in Table 2. Mutation in codon 12 of K-ras gene was observed in 15 of 57 (26%) mucinous tumors, but in 1 of 47 (2%) serous ones. The higher frequency of K-ras mutation in mucinous tumors than in serous ones is statistically significant ($P < 0.0005$; Fisher exact test). In mucinous tumors, the statistical difference of the incidence of K-ras mutation was found between adenomas (4 of 30) and carcinomas (7 of 15) ($P < 0.03$).

In mucinous adenomas, mutation in codon 12 of K-ras gene was associated with the lining of the epithelium. The incidence of K-ras mutation in mucinous adenoma was significantly higher in intestinal type (4 of 13) than in endocervical type (0 of 17) ($P < 0.03$). However, in mucinous tumors of borderline malignancy, a higher mutation frequency was observed in endocervical type (2 of 5) compared to intestinal ones (2 of 7), but this was not statistically significant ($P = 0.15$) (Table 2).

**Discussion**

The present study showed a higher incidence of mutations in codon 12 of K-ras gene was associated with intestinal type-mucinous adenoma. This correlation was not observed in mucinous tumors of borderline malignancy. The origin of intestinal-type mucinous tumors is considered as a teratoma. The finding of goblet cells, argentaffin cells, Paneth cells, and cystic teratomas, which are present in about 5% of mucinous tumors, suggests that some mucinous neoplasms may represent monomorphic endodermal differentiation of a teratoma (7). In this study, all the mucinous adenomas with a mutated K-ras gene were classified as intestinal type. Therefore, mutation of K-ras gene may be involved in tumorigenesis of intestinal-type mucinous adenomas originating from teratomas. K-ras mutation was not found in endocervical type of adenomas, but was found in both intestinal type and endocervical type of tumors of borderline malignancy. Mutations of K-ras might be involved in the development of endocervical-type mucinous tumors of borderline malignancy. The genetic events including K-ras mutation are possibly associated with the determination of histological subtypes during ovarian tumor development.

Our study is the first comprehensive analysis of K-ras mutation in ovarian mucinous adenomas. The higher incidence of K-ras mutations in ovarian mucinous tumors of borderline malignancy and carcinomas than in serous ones is consistent with previous reports (4, 8). The rate of positive cases (2%) in serous carcinomas was lower than that reported previously (20–30%) in serous carcinomas (4, 8). Serous carcinomas with mutated K-ras gene reported previously have all been in stage III or IV (4, 8), but in our study, the mutation-positive case was in stage Ia, and no K-ras gene mutations were found in 10 cases of stage III and in 2 cases of stage IV serous carcinomas. The reason for this discrepancy remains unknown, but racial differences might be one possibility.

Recent studies revealed that the ras family of protooncogenes has been involved not only in cellular carcinogenesis but also in control of cellular growth and differentiation (9). In certain human neoplasms, K-ras gene mutations have been suggested to play a role in the cellular pathway of mucinous differentiation (10, 11). In our study, K-ras mutations were preferentially identified in mucinous carcinomas, tumors of borderline malignancy, and adenomas compared to serous ones. These results suggest that the K-ras mutations are an early genetic event in a subset of ovarian mucinous tumors.

In colorectal tumors, K-ras mutations are detected at a similar incidence in both adenomas and carcinomas (2, 12). In lung adenocarcinomas, alterations in codon 12 of K-ras gene occur early and irreversibly during their development (13). In gynecological tumors, the incidence of K-ras mutations in endometrial hyperplasias is similar to the incidence in carcinomas (14). These findings suggest that
These results indicate possible pathways, other than (P = 0.15). On the other hand, in ovarian tumors, a lower incidence of K-ras mutation in serous adenomas compared to mucinous adenomas was observed in our study, and also in a previous report (15). These results indicate possible pathways, other than K-ras gene activation, in the development of ovarian neoplasms. More detailed studies are required to define the ovarian adenoma-carcinoma sequence, and to clarify other genetic alterations in mucinous ovarian carcinogenesis.

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References

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