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 tumors and carcinomas 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.

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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 codons 12 and 13, and codon 61 of K-ras gene were amplified by using oligonucleotide primers as follows: 5’-GGCCTGCTGAAAATGACTGA-3’ (forward) and 5’-ATTGTCCTCAACAAATGATTCC-3’ (reverse) for codons 12 and 13, 5’-TTCCTACAGGAAGCAAGTAG-3’ (forward) and 5’-CAGAAAAGGCCCCCTCCCA-3’ (reverse) for codon 61. After 40 cycles of PCR (93°C, 57°C, and 72°C for 1, 1, and 1.5 min, respectively) from 1 μg of sample DNA in a 5-μl volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.75 mM MgCl2, 0.02% gelatin, 0.05% Tween 20, 25 μM concentrations of each deoxyribonucleotide triphosphate, 0.125 unit of Taq DNA polymerase (Wako, Osaka, Japan), 0.02 pmol of each primer, and 2 μCi of [α-32p]dCTP (3000 Ci/mmol, Amersham, United Kingdom); the PCR products were analyzed by a 6% polyacrylamide gel with or without 5% glycerol at 25°C. Autoradiography of the gel was analyzed with a Fujix BAS2000 Imaging Analyzer (Fuji Photo film, Tokyo, Japan).

Sequencing of PCR Products. The direct sequencing of PCR products was performed by using a double-stranded DNA Cycle Sequencing System (GIBCO BRL, Gaithersburg, MD). The excess primers were removed by centrifugation, using Suprec-02 (Takara Biomedicals, Kyoto, Japan). The primers used for PCR-SSCP analysis were end-labeled with γ-[32p]ATP (5000 Ci/mmol, Amersham) and used for sequencing. The K-ras mutations were confirmed by sequencing both strands in an 8% polyacrylamide gel containing 7 M urea.

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 bor-
deline 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
deline malignancy, and carcinomas represented the wild-type pat-
tern. In all cases examined, no altered migration indicating the pres-
ence 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 con-
cordance 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 includ-
ing adenomas compared to serous tumors was observed. K-ras mu-
tations were detected in stages Ia and Ib of mucinous tumors includ-
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tations 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 IIC 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 fre-
quency 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 ad-
ena. 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 adeno-
mas 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 in-
cluding 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, tu-
mors 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 ade-
carcinomas, 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 simi-
lar to the incidence in carcinomas (14). These findings suggest that

K-RAS MUTATION AND HISTOLOGICAL TYPES OF OVARIAN TUMORS

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represent monomorphic endodermal differentiation of a teratoma (7).
In this study, all the mucinous adenomas with a mutated K-ras gene

Fig. 1. PCR-SSCP analysis of K-ras gene codon 12 and 13 mutations in human ovarian
tumors. Top ordinate, case numbers, N, normal human placenta. Arrowheads, bands
showing mobility shifts.

Fig. 2. Mutations in K-ras gene codon 12 in human ovarian tumors. N, normal human
placenta. Numbers correspond to the case numbers in Table 1. From top to bottom of the
gel, the sequences correspond to the 5' to 3' direction in the transcribed strand. Mutations
were confirmed by sequencing of both strands.
the K-ras gene may play an important role in an early step of carcinogenesis. On the other hand, in ovarian tumors, a lower incidence of K-ras mutation in serous adenomas compared to mucinous ones 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 ovary adenoma-carcinoma sequence, and to clarify other genetic alterations in mucinous ovarian carcinogenesis.

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**References**


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